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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ENTOMOLOGY—BULLETIN No. 98.
L. O. HOWARD, Entomologist and Chief of Bureau.

HISTORICAL NOTES ON THE CAUSES OF BEE DISEASES.

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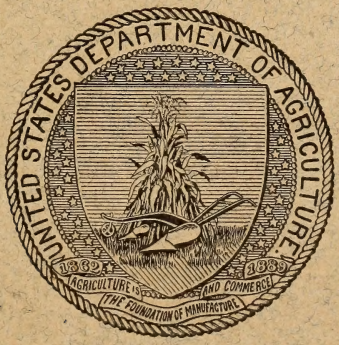
BY

E. F. PHILLIPS, PH. D.,
In Charge of Bee Culture,

AND

G. F. WHITE, PH. D., M. D.,
Expert in Bacteriology.

ISSUED MARCH 26, 1912.



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Pearl H. Garrison, *preparator.*
H. A. Surface, D. B. Casteel, *collaborators.*

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Entomology,
Washington, D. C., September 27, 1911.

Sir: I have the honor to transmit herewith a manuscript entitled "Historical Notes on the Causes of Bee Diseases," prepared by Drs. E. F. Phillips and G. F. White, of this bureau. The investigations of the causes of bee diseases are highly important in the control of these maladies. Many of the papers on this subject are not available and many also record errors in observations and conclusions. The purpose of the present paper is to assist bee keepers in obtaining a proper understanding of the work done by the various investigators whose papers are discussed. I respectfully recommend the publication of this manuscript as Bulletin No. 98 of this bureau.

Respectfully,

L. O. Howard,
Entomologist and Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

	Page.
Introduction	11
American foul brood	11
European foul brood	12
The so-called "pickled brood"	12
Paralysis	13
Dysentery	13
Isle of Wight disease	13
Consideration of papers on the causes of bee diseases	13
Schirach, 1771	13
Leuckart, November 12, 1860	14
Molitor-Mühlfeld, April 15, 1868	14
Preuss, October 1, 1868	15
Schönfeld, November 15, 1873	16
Dzierzon, 1882	18
Cheshire, August 1, 1884	18
Cheshire, August 15, 1884	21
Cheshire, September 1, 1884	22
Cheshire, September 15, 1884	24
Cheshire, October 15, 1884	25
Cheshire and Cheyne, August, 1885	25
Cheyne, August, 1885	29
McLain, 1887	35
McLain, 1888	37
Lortet, February, 1890	38
Mackenzie, December, 1892	39
Howard, March 1, 1894	41
Howard, September 10, 1896	42
Howard, February 15, 1900	44
Harrison, December, 1900	48
Lambotte, September 25, 1902	53
Harrison, February 28, 1903	56
Moore and White, January 15, 1903	58
White, January 15, 1904	61
Bahr, 1904	64
Burri, October and November, 1904	64
White, January 14, 1905	66
White, June, 1905	67
Wilson, 1905	67
Burri, January, 1906	68
Maassen, June, 1906	72
Maassen, June, 1906	73
Bahr, 1906	74
Phillips, October 3, 1906	75
Erne, November, 1906	75

Consideration of papers on the causes of bee diseases—Continued. Page.
White, November 6, 1906 76
Maassen, February, 1907 78
Imms, June, 1907 79
White, July 29, 1907 80
Phillips, December 31, 1907 82
Maassen, 1908 82
Maassen, September, 1908 85
White, December 26, 1908 85
Malden, February, 1909 87
Zander, August, 1909 89
Maassen, March, 1910 91
Maassen and Nithack, March, 1910 92
Malden, June, 1910 92
Zander, 1910 93
Zander, 1911 93
Brief chronological summary of the work on the causes of bee diseases 93
The different diseases that attack bees 93
The causes of bee diseases 94
Index 95

PREFACE.

Bees, like many other members of the animal kingdom, are known to suffer from diseases. Simultaneously with the good work that has been done during the last half century toward the determination of the causes of the various diseases of man and animals, there has been some work done on the causes of bee diseases. This work has caused considerable literature to be written on the subject. Although this literature contains much that is valuable, it abounds in statements that are erroneous and in conclusions that seem unjustifiable. Many of the inaccurate statements and conclusions have been frequently copied in the past and they are still too often copied into the current literature on bee diseases. The bee keeper, therefore, in reading is often at a loss to know what is true and what is untrue; what is actually known and what is not known.

For the purpose of aiding the bee keepers with this literature, we have reviewed here portions of several original papers dealing with the causes of bee diseases. It is hoped that this bulletin may serve as a means whereby the bee keeper may solve for himself some of the apparent mysteries found in beekeeping literature.

In selecting the papers for review, for the most part, those were chosen which were written by men who had worked more or less on the causes of bee diseases. The reviews that have been made contain the more important beliefs concerning the causes of these diseases that were entertained by the authors of the different papers at the time they wrote. The classification of the diseases of bees as understood by these different men is also frequently included. The original papers naturally contain much that has not been mentioned in these brief reviews, and therefore the reader is urged, if opportunity permits, to read the papers cited in this bulletin rather than the reviews. It is probable that the papers here considered might with profit have been more completely reviewed and that other papers might with profit have been considered, but if either had been done it is probable that the length of the bulletin would have defeated its object.

It is hoped that the readers of bee-disease literature will learn, so far as possible, to judge correctly an article that discusses in any way the causes of bee diseases. To do this, one should first of all learn who are actually doing work on the causes of these diseases.

The writings of all these men should be read. If an investigator has done work on the causes of other diseases than bee diseases, but chooses to write on bee diseases, the reader will usually profit by reading his papers. The great mass of literature, on the other hand, created by those who have not worked on the cause of any disease can as a rule with profit be rejected.

Having determined whose papers should be read, the character of the work of each investigator should be carefully noted. If the character of a man's work proves to be good, give weight to all his statements, but if the character of a man's work is poor, expect untrue statements and erroneous conclusions. If one will learn in this way to judge the different papers, one will soon know what to believe and what to suspect, but if one does not learn to do this he will be forever at the mercy of printed pages.

As the reader forms his opinion of the character of the work done by the different men referred to in this bulletin, permit the suggestion that he exercise some leniency inasmuch as the time at which a man works and the circumstances under which he labors are frequently in a measure responsible for mistakes. The reader will note, however, that many times the mistakes made in the study of bee diseases have been made only because insufficient and careless work was done by the investigator. In such cases no leniency is to be exercised in arriving at conclusions.

The writers of this bulletin have commented very little on the character of the work done by the different authors of the papers reviewed. The views of these men as they are found in the papers are given and the reader is allowed and urged to judge for himself whether or not such views are true. To aid the reader, however, the writers have made a few suggestions when it was thought that they might prove advantageous. The page references refer to pages in this bulletin.

In reading a paper there is always the danger of misinterpreting an author's conception. This danger is greatly increased if the author of the paper criticized uses a foreign language. Realizing this possible source of error, we have endeavored in every case to be cautious. When quotations from papers written in a foreign language were selected, rather free translations of them into English have been made.

We disagree with a very large number of the statements which have been made by different authors referred to in this bulletin concerning the causes of bee diseases. Therefore let it be emphasized that the reviews which are here made are intended to express the opinion of the author of the paper reviewed, and not by any means the opinion of the writers of this bulletin.

To entomologists who feel an interest in the causes of insect diseases and who wish to be able to judge with some satisfaction the work that has been and is being done on insect diseases this bulletin will be of special interest. It is believed that by the learning of the mistakes made by workers on bee diseases, and by the learning of the causes for such mistakes, the careful reader will be enabled to judge more accurately the value of the various reports that appear on the diseases of insects.

THE AUTHORS.

HISTORICAL NOTES ON THE CAUSES OF BEE DISEASES.

INTRODUCTION.

Bee keepers, as a rule, manifest a keen desire to know about the causes of bee diseases and they show a lively interest in the investigations leading to the determination of the causes. This is gratifying to those working on these diseases and will be a great benefit to the apiarist who must treat the diseases. The losses to apiculture from diseases are enormous, and inasmuch as the successful treatment of a disease depends largely upon a knowledge of the cause of the disease to be treated it behooves every owner of an apiary to become as familiar as possible with the causes of bee diseases.

The facts that are known about the causes of bee diseases unfortunately are altogether too few. As this can be said of all diseases affecting the animal kingdom, the bee keeper has no cause for despair. An attempt, however, will be made in this bulletin to furnish data from which the bee keeper may be able to inform himself concerning the facts that are really known about the causes of bee diseases.

In this introduction it might be well to classify the bee diseases as the writers of this bulletin understand them. Bee diseases can be conveniently classified under those affecting the brood and those affecting the adult bee. The most important brood diseases are American foul brood, European foul brood, and the so-called "pickled brood." The disorders affecting adult bees that are of most importance are being referred to at present under the names of paralysis, dysentery, and Isle of Wight disease.

American foul brood.—American foul brood is a very widely distributed disease and better known to bee keepers than European foul brood. It is the one which is generally referred to by the bee keeper at the present time when he speaks of "foul brood." The brood affected with this disease is usually capped before it dies. The color of the dead brood presents in general various shades of brown. The marked ropiness of the decaying remains of the dead larvæ is probably the most characteristic and well-known feature of the disease. The punctured cappings, the scales formed from dried-down larvæ, and the disagreeable odor sometimes present are aids to its diagnosis. This disease is clearly an infectious one. The exciting cause of it is a bacterium known as *Bacillus larvæ.*

European foul brood.—European foul brood is the disease which Cheshire and Cheyne (p. 25) described in their studies of foul brood. Howard (p. 44), of Texas, made a very brief and unsatisfactory study of this disease at one time and named it "New York bee disease" or "black brood." We are strongly inclined to believe that Burri (p. 68) was working with this disease for the most part during his study of the condition which he refers to as "sour brood." European foul brood is less widely distributed in this country than is American foul brood. In European foul brood one finds, as a rule, most of the diseased brood as yet uncapped. In general, the brood dead of this disease presents various shades of yellow. Usually there is no ropiness; at times, however, there is. That degree of ropiness, however, which is so characteristic of American foul brood is seldom present in European foul brood. There is frequently a slightly sour odor to the diseased brood. The rapidity with which this disease spreads in a new territory and the marked destructiveness of it are features which most bee keepers have experienced who have been so unfortunate as to have the malady affect their apiary. The disease is clearly, therefore, an infectious one. The exciting cause is not known. Claims are made by some that certain species of bacteria stand in direct etiological relation to the disease, but satisfactory evidence to prove such contentions are wanting.

The so-called "pickled brood."—Howard (p. 42), of Texas, described what he chose to call pickled brood. His findings have never been confirmed. The name "pickled brood," however, is very frequently used by bee keepers in referring to a diseased condition of the brood. Howard's description of "pickled brood" (p. 43), however, does not apply to such a condition. Since the name "pickled brood" is not accurately applied and is, moreover, entirely inappropriate for the condition which we find, we prefer for the present to use the expression "so-called pickled brood." In this condition the brood dies about the time of capping. The body wall of the larva, in a case which might be called typical, is intact and rather tough. When this wall is broken, one often finds a watery content in which is suspended a granular substance. As a rule a very small proportion of the brood is affected. The disease does not seem to be infectious. The loss to the colony in comparison with European foul brood and American foul brood is slight. This disorder, therefore, should arouse no great amount of fear. While the number of colonies lost from this disease is comparatively small, in the aggregate many bees die as a result of the condition. The disease has a very wide distribution. The exciting cause is not known.

There is very little that is definitely known about the diseases of adult bees. They have not been sufficiently investigated to make it possible to classify them with any degree of satisfaction.

Paralysis.—But little is definitely known about paralysis of bees. The disease has not been demonstrated to be infectious. Many suppositions have been made by different writers as to the cause of the trouble, but no satisfactory evidence has been produced to prove the cause.

Dysentery.—A condition known as dysentery has often been observed by the bee keeper. But little is known about the disorder. There is considerable evidence that the nature of the winter food plays a part in its causation. Zander (p. 89) has recently suggested that there are two forms of this affection, a noninfectious one and an infectious one. To an infectious form he ascribes *Nosema apis* as a cause. Much work must yet be done upon this condition.

Isle of Wight disease.—The disorder known as Isle of Wight disease was first reported from the Isle of Wight by Imms (p. 79). Malden (p. 93) reports that the disease has more recently spread to the mainland (England). This disorder has so far not been found in any other country. The cause has not been definitely established.

It is urged that the reader peruse the preface to this bulletin (pp. 7–9) carefully. By so doing the intent of the writers of this bulletin will be better understood and the chances of misinterpretation will be lessened.

CONSIDERATION OF PAPERS ON THE CAUSES OF BEE DISEASES.

SCHIRACH, 1771.

Schirach[1] in 1771 classified the diseases which most frequently attack bees as follows: (*1*) Dysentery; (*2*) disease of the antennæ; (*3*) foul brood; (*4*) queens laying drone eggs only; (*5*) sterile queen; (*6*) queenless colonies.

Dysentery he considered to be dietary in origin. No belief is expressed as to the cause of the disease of the antennæ, to which he refers, but he states that with this disease the danger is not great. The disease which he designates as foul brood, however, he believed to be quite dangerous, very fatal, and a true pest after it has reached a certain stage. To this condition he attributed two causes, one cause being ascribed to the improper food which was consumed by the larvæ, and the other being a fault of the queen in permitting the brood to be so arranged in the cells that the heads point inward. Considering these two widely different causes ascribed to an abnormality in the brood, one might suspect that there was more than one disease in the condition which he designated as foul brood. That part of the disease condition, to which as a cause he ascribed the food, could well be an infectious disease—either American foul

[1] Schirach, A. G., 1771. Histoire naturelle de la reine des abeilles, avec l'art de former des essaims. La Haye. Pp. LXIII+269; 3 plates.

brood or European foul brood. The other form of the disease, in which the brood was supposed to be placed with the head directed inward, most probably was not an infectious disease. In the treatment of foul brood Schirach recommends the removal of all combs from the bees. This principle is the one upon which is based the methods which are most successful at the present time in the treatment of the infectious brood diseases.

The other abnormalities in the colony which are mentioned in the paper relate to the condition of the queen. These are conditions familiar to the bee keeper, but which may occur more often when an infectious brood disease is present. Mention is also made of the fact that brood is sometimes killed by chilling. Schirach refers to this as an accident and not as a disease.

Leuckart, November 12, 1860.

Leuckart [1] had entertained the opinion that infectious foul brood was due to a fungus, and he felt that his view was strengthened by some work which was done on the diseases of the silkworm. During the summer of 1860, however, he had an opportunity to see much infectious foul brood in samples of comb and in colonies. In the diseased material he found no fungi that he could not attribute to the phenomenon of decay. He states in the paper that foul brood is obviously a collective name that includes various forms of disease with the features in common of being epidemic, attacking early stages, and being usually fatal. One sample was examined, and a number of diseased and dead larvæ was found to contain an unidentified fungus. The majority of them, however, did not contain the fungus; yet these latter larvæ were thought to be dying of the usual type of foul brood. From his summers' experiences Leuckart arrived at the conclusion that the infectious foul brood was not due to a fungus.

Molitor-Mühlfeld, April 15, 1868.

Molitor-Mühlfeld [2] in 1868 reported some startling observations relative to the cause of foul brood. He writes that foul brood is of two kinds, the mild kind and the so-called infectious or virulent one. The mild form of foul brood, according to his views, resulted from a chilling of the brood. During the early warm days of spring, he argues, brood rearing is stimulated to such an extent that when colder weather follows it is impossible for the bees to care for all the brood, and as a result the neglected brood is chilled, dies, and be-

[1] Leuckart, Dr., November 12, 1860. Zur Naturgeschichte der Bienen. 3. Zur Kenntniss der Faulbrut und der Pilzkrankheiten bei den Bienen. Eichstädt Bienenzeitung, 16 Jahrg., Nro. 20, pp. 232-233.

[2] Molitor-Mühlfeld, April 15, 1868. Die Faulbrut, ihre Entstehung, Fortpflanzung und Heilung. Eichstädt Bienenzeitung, 24 Jahrg., Nro. 8, pp. 93-97.

comes foul. From this condition, this author stated, no danger is to be feared, as the bees afterward remove all this dead brood, leaving the colony free from danger. The cause of the virulent form of foul brood is attributed by Molitor-Mühlfeld to a small parasitic ichneumon fly, reddish-yellow in color and scarcely one-sixth of an an inch long, to which he gave the name *Ichneumon apium mellificarium*. He writes that this fly had already been observed about foul-brood colonies by another writer, but that it was thought to be a carrion fly. Concerning the life history of these flies, he says that they press into the hives and lay their eggs in the bee larvæ. The larvæ live in spite of this until the cell is capped and the cocoon is spun. During this time the fly larvæ feed upon the fat of the bee larvæ, and finally bore their way out of the body into the cell, undergo metamorphosis, and in a few days escape from the cells through openings which they make in the center of the cell-capping. These young adult flies now mate, sting other bee larvæ, lay their eggs, and continue the cycle. The time which elapses from the egg of this parasitic insect to the adult is given as about from 10 to 12 days. This, to his mind, explained the rapid increase of the exciting cause of foul brood. As a result of the parasitic existence of this fly in the bee larvæ, these larvæ die and change into a ropy, sticky, ill-smelling mass which the bees can not remove.

Furthermore, he argues that if instead of the diseased larvæ dying, as they do, after capping, they should die before this stage was reached, then the dead bodies would be removed early and with them the larvæ of the fly; but since the brood is always capped before death takes place the capped cells afford a protection for the parasitic insect until it becomes an adult ready to emerge.

In making a diagnosis, it is stated, the cell-cappings should be examined, and if they are punctured then the disease is positively the infectious foul brood. As a treatment for the infestation of the brood by this insect in a colony in which infectious foul brood already exists, it is recommended that the combs be removed to a clean hive with new foundation, and that the treated colonies and other colonies in the apiary be protected by pouring at frequent intervals camphor dissolved in oil of turpentine, between the hives in the yard and also sometimes on the alighting boards. This is done to prevent, by the odor of the turpentine and the camphor, the entrance of the ichneumon fly into the hives.

PREUSS, OCTOBER 1, 1868.

In a paper written by Preuss,[1] in 1868, his views on the causes of foul brood are given. The distinction which he would make between

[1] Preuss, Dr., October 1, 1868. Das Wesen der bösartigen Faulbrut besteht in einem mikroskopischen Pilze, *Cryptococcus alvearis*. Sie kann verhutet und geheilt werden. Eichstädt Bienenzeitung, 24 Jahrg., Nro. 19 u. 20, pp. 225-228.

mild and virulent foul brood is, that virulent foul brood is caused by a fungus which he named *Cryptococcus alvearis*, and that the mild foul brood is due to some other cause. His conclusion concerning the virulent foul brood was reached through a microscopic study of foul-brood material. Preuss had been somewhat familiar with bee-keeping since early boyhood, and had had the opportunity of visiting numerous apiaries in the Vistula Valley, but had not encountered foul brood until in 1866, when a friend had called his attention to the disease in an apiary of the latter in which he was using the Dzierzon hives. Preuss immediately undertook the investigation of the character of the disease by studying microscopically the larvæ which had died of the disease. A small bit of the dead larvæ was added to a little water, covered with a glass, and studied in the fresh condition. Numerous spherical bodies measuring 2 μ in diameter were seen and identified by him as belonging to the genus Cryptococcus, to which he gave the name *Cryptococcus alvearis*. Larger objects which were present were recognized as fat bodies.

Very nearly related to this organism, Preuss writes, is a fungus that causes fermentation, *Cryptococcus fermentum*. It was his belief that if this latter species infected or fell upon a larva it might, under favorable temperature and moisture conditions, change into *Cryptococcus alvearis* and in this way produce foul brood. Practical bee keepers had, prior to this time, emphasized the danger of foul brood transmission by the feeding of fermented honey. One bee keeper of large experience had attributed foul brood to meal feeding, and since meal is a good medium for the growth of fungi, Preuss was inclined to favor the view. He argued that since the fungus of fermentation is widespread in nature, the brood dying from cold or neglect of any kind may constitute a fruitful soil in which this fungus could grow and thus become the cause of infectious foul brood. Medication in the treatment of the disease Preuss held to be quackery and recommends instead the removal of the diseased frames from the hive, but not the destruction of the hives. The hives were to be washed with 10 per cent sulphuric acid, followed by water, and afterwards put into an oven and heated to the boiling temperature for some hours. The frames containing diseased material were to be burned, and those frames which were free from such material were to be used again. All dead bees were to be buried, as they might become a source of fungous growth, and the ground in front of the hive was to be sprinkled with sulphuric acid and then dug up deeply.

Schönfeld, November 15, 1873.

In the absence of conclusive experimental proof, the theories advanced by Preuss in the paper just considered were not univer-

sally accepted. Schönfeld,[1] therefore, set about to supply incontrovertible evidence to prove the cause of infectious foul brood. He received a small mass of decaying larvæ about the size of a pea and placed it under an inverted funnellike apparatus. An opening for the admission of air was made from below; the exit was an opening above in which was placed a stopper made of cotton. Placing this apparatus near the window, that it might receive the heat of the sun, he hoped, by the current of air which would thus be produced, to collect on the cotton, filling the exit, the spores of the fungus which would be floating off in the air from the foul-brood mass. Upon examining the cotton he found what he supposed was the fungus in the form of a micrococcus. This was the first part of his experiment. In the second part of it he used this cotton to infect healthy larvæ. Four square inches of brood was covered by a layer of cotton. The cotton was taken from one of the stoppers that had been contaminated with the fungus by means of the apparatus. After two unsuccessful trials he made a third attempt, which was considered by him as being successful. After a lapse of four days seven larvæ had died and numerous micrococci were found in their dead bodies.

In another experiment the same author used the larvæ of the blowfly (*Musca*), *Calliphora vomitoria.* Some cotton contaminated in the manner outlined in his first experiment was placed upon some meat upon which these larvæ were feeding. Nine days after adding his supposed virus he found dead larvæ which upon microscopic examination revealed to him again the presence of numerous micrococci. The results of these experiments convinced him that this micrococcus was the cause of infectious foul brood, and he believed that the fact would be accepted without question.

The experiments of Schönfeld were not, however, universally accepted as conclusive. This induced him to perform other infection experiments. This time he used caterpillars of (*Pieris*) *Pontia brassicæ* and (*Pieris*) *Pontia rapæ.* The virus was mixed in distilled water and painted on the exterior of the insect, with the result that those so treated died while the checks developed normally to healthy pupæ. Microscopically, however, the check caterpillars showed also the presence of the fungus. This caused him to doubt somewhat his conclusions relative to the blowfly experiment. He believed, however, that sufficient evidence had now been produced to justify the conclusion that infectious foul brood is a mycosis and that the fungus *Cryptococcus alvearis* is the exciting cause of the disease.

[1] Schönfeld, Dr., November 15, 1873. Faulbrut-studien, Pt. I. Eichstädt Bienenzeitung, 29 Jahrg., Nro. 21, pp. 250–254; January 15, 1874. Faulbrut-studien, Pt. II, Eichstädt Bienenzeitung, 30 Jahrg., Nro. 1, pp. 3–5.

Dzierzon, 1882.

For many years Dzierzon and others entertained the belief that there existed two forms of foul brood, a mild form and a virulent one. In his "Rational Bee Keeping," Dzierzon[1] has written the following concerning the kinds of foul brood.

> There is one kind that is mild and curable, and another kind malignant and incurable; both kinds are, however, contagious.
>
> The curable occurs in this way: More of the larvæ die still unsealed, while they are still curled up at the bottom of the cell, rotting and drying up to a grey crust, that may be removed with tolerable ease. The brood which does not die before sealing mostly attains to perfection, and it is only exceptionally that individual foul-brood cells are met with sealed.
>
> This is exactly reversed in the malignant kind of foul brood. In this the larvæ do not generally die before they have raised themselves from the bottom of the cell, have been sealed and begun to change into nymphs. The rotten matter is, therefore, not found on the cell floor, but on the lower cell wall; it is brownish and tough, and dries up to a firm black crust, both in consequence of the heat prevailing in the hive, and of a small opening bitten in the depressed cover. This matter the bees are not able to remove; and when they are in some strength, they can at most get rid of it by entirely biting down the tainted cells and making fresh ones.

The description which Dzierzon here gives of the "mild" form of foul brood applies very well to European foul brood, and his description of the "malignant" form applies equally well to American foul brood. It is fair to suppose that he encountered both European foul brood and American foul brood, but instead of recognizing them as two distinct diseases, he thought them to be two forms of the same disease.

Cheshire, August 1, 1884.

The work of Cheshire on the cause of bee diseases is of much interest and should be somewhat carefully considered, inasmuch as it has directly and indirectly caused much confusion in the minds of bee keepers concerning the nature, cause, and treatment of foul brood.

The first paper[2] by him to be considered was the outgrowth of an invitation by a committee of the British Bee Keepers' Association about the last of May, 1884, to give an address before the association on foul brood. A paper of considerable length was prepared and was read before that body of bee keepers on July 25, 1884. In this address the subject of foul brood was taken up under three separate headings: (1) "The nature of foul brood as a germ disease;" (2) "The means of the propagation of the disease;" (3) "The method of

[1] Dzierzon, Johannes, 1882. Dzierzon's Rational Bee Keeping; or the theory and practice of Dr. Dzierzon. Translated from the latest German edition by H. Dieck and S. Stutterd. Edited and revised by Charles Nash Abbott, London. Pp. xvi+350.

[2] Cheshire, Frank R., August 1, 1884. Foul brood (not Micrococcus, but Bacillus), the means of its propagation and the method of its cure. British Bee Journal, Vol. XII, No. 151, pp. 256–263.

cure." As Dzierzon and others had done, Cheshire refers to two forms of foul brood. Concerning the appearance of these two forms of the disease, he writes as follows:

> The appearance of foul brood is undoubtedly familiar to almost all before me. A larva, if attacked early, begins to move unnaturally, and instead of lying curled round on the base of the cell frequently turns in such a way as to present its dorsal (back) surface to the eye of the observer. A little attention will then show that the colour of the larva is inclined to yellow instead of being pearly white. Such grubs are only rarely sealed over. Those more advanced before the disease strikes them are in due course sealed, but death overtakes them, their bodies become brown and fœtid, and the sealing sinking gets pierced by an irregular hole. From this may be gathered the general indications of the disease, which is usually accompanied by very energetic fanning at the hive mouth, from which in advanced cases an indescribable and nauseating odour is emitted. The larvæ and chrysalids dead of the disease dry up to a coffee-coloured, tenacious mass lying at the bottom of the cell, so tenacious, indeed, that it may be drawn out into long threads like half-dry glue. The drying process completed, a blackish scale is all that remains.

Cheshire gives here a description of the disease very similar to the one found in Dzierzon's writings. His description of the disease when it attacks the larvæ early in the developmental stage fits quite well that of European foul brood; and the description which is made of the brood which is attacked later in the stage of development is equally accurate for American foul brood. There is little room for doubt that the two forms of foul brood described by Dzierzon and Cheshire are the two distinct diseases, European foul brood and American foul brood.

Cheshire began his study of the disease by examining the juices of healthy larvæ microscopically. In these he found no bacteria. He then examined the coffee-colored, foul-broody, dead larval mass and observed numerous ovoid bodies which he demonstrated later to be the spores of bacteria. This led him to suspect that Schönfeld had fallen into the error of supposing that these spores were micrococci. Having found numerous spores in the remains of larvæ which had been dead for a long time, he examined some larvæ which were dead but in a fresher condition. In these he observed many rodlike bacteria and fewer spores. He next examined larvæ which showed signs of disease, but which were yet alive, and found their juices to be filled with actively motile rods. These rods were sometimes arranged in chains—leptothrix forms. He now examined larvæ representing the different stages of the disease, and observed: First, that in the beginning of the attack, rods only were seen; second, as the disease advanced, the rods began to form spores; third, as the larvæ assumed the viscid state, spores were very rapidly formed; and fourth, in a few days only spores in very large numbers were found. These observations on the morphology of the bacillus are good, but the conclusion which he drew from them, "Foul brood, then, is a bacillus disease," is of course unwarranted.

Cheshire, believing that foul brood was due to a bacillus and not to a micrococcus, as claimed by Schönfeld, sought to demonstrate the fact by repeating the inoculation experiments of the latter, using the larvæ of the blowfly used by Schönfeld (*Musca*) *Calliphora vomitoria*. Sixty blowfly larvæ were divided into three equal groups: 20 were not brought near foul-brood material; 20 were inoculated with the bacillus in the vegetative form; and 20 with the spores of the bacillus contained in the coffee-colored foul-brood material. Microscopic examination at the end of 24 and 48 hours failed to give evidence of disease in the fly larvæ. After 72 hours, however, active bacilli were observed. Cheshire writes, "This is most completely confirmatory of my position; but how could it be reconciled with Schönfeld's assertion, that he found the dead flies full of micrococci? Had he searched further, he would have discovered that dead blowflies are generally full of micrococci." In demonstrating this error of Schönfeld, he unfortunately made an error quite as great himself.

Cheshire attempted to obtain, by cultures, proof to support his contentions concerning the etiology of foul brood. He prepared a medium by taking drone larvæ and expressing and straining their juices into two test tubes. Tube No. 1 was inoculated with a small quantity of coffee-colored material, which for the most part contained only spores; tube No. 2 was inoculated with a trace of fluid from a diseased larva which contained the vegetative form of the bacillus. These tubes were then suspended in a hive between the frames in order that the temperature for growth might be right. After a period of 22 hours an examination was made. Observing practically no spores and many bacilli in tube No. 1, and many bacilli in tube No. 2, he reached the conclusion that many of the spores introduced into No. 1 had germinated, and that the bacilli introduced into No. 2 had increased by multiplication. From this he concludes that the rods were produced from the spores when suitable conditions permitted the germination of the latter, and that the rods produced the spores when the reverse conditions were present. From the technique used, of course, the data he obtained could be of very little value. Thus Cheshire's culture experiments failed as completely in demonstrating the etiology of foul brood, as did his experiment with blowfly larvæ. The experiment, however, is of some interest as it is among the first cultural work done on bee diseases, and also because here larvæ of bees were used as a medium.

Aside from the larvæ, Cheshire suspected that adult bees suffered from foul brood. He was of the opinion that if two colonies, a healthy and a diseased one are selected for observation, and 5,000 larvæ be removed from the healthy one, and 1,000 larvæ die of disease in the diseased one, that the healthy colony will progress pretty much as though it had lost nothing, while the diseased one will, as a rule,

diminish very perceptibly in strength. This difference in the strength of two such colonies suggested to Cheshire the probability that the adult bees died from foul brood.

In an attempt to settle this question he went to a foul-brood colony and observed one bee dead, another hopping in abortive flight, and finally a third and fourth worn out. The microscopic examination of the first bee was negative, but the second bee was full of active bacilli. This, he believed, was sufficient to answer the question. From this he concluded that workers and drones suffer from the disease, which suggested to him the possibility that the queen suffers also and, if the queens suffer, he says, why are the eggs not also affected? As a result of these observations he suggested that the name foul brood is inappropriate, since as he supposed, the disease affects adults as well as brood.

At this point in his paper Cheshire gave to the bacillus which he saw the name *Bacillus alvei*, meaning bacillus of the hive. This name he claims represents both generically and specifically what the disease really is.

In the treatment to combat the disease he recommended the feeding of phenolated sirup, in the proportion of 1 part pure carbolic acid (phenol) to 500 parts sirup. This drug had been used, however, in the treatment of bee diseases before Cheshire recommended it. In expressing his apparent confidence in carbolic acid as a cure for foul brood, he writes:

> I could take an apiary beginning of March with every stock diseased, and by May 1, with but very little labour, deliver it up clean and strong, as strong as though the disease had never appeared.

Naturally many practical bee keepers who had had experience with foul brood hesitated to accept literally such a broad statement.

Cheshire, August 15, 1884.

The idea which many bee keepers have that a queen with diseased ovaries will transmit the disease to the brood is largely based upon the writings of Cheshire.[1] In a paper he relates his observations in support of his belief that the queen may be responsible for foul brood in a colony. He received from a bee keeper a queen, nearly dead, that was taken from a colony in which some of the larvæ seemed to die immediately after hatching. One ovary from this queen, which was yellow and soft, was removed. A portion was examined under a microscope, and four or five bacilli were observed. Detaching now a half-developed egg, it was placed in a little water upon a slide and covered with a cover-glass. Upon examination, no less than nine bacilli were seen. The right ovary, it is stated,

[1] Cheshire, Frank R., August 15, 1884. Queen and eggs containing *Bacillus alvei*—foul brood (?) British Bee Journal, Vol. XII, No. 152, pp. 276-277.

was nearly free from disease. After a long search only two or three bacilli were found in this latter ovary. Cheshire now believed, apparently, that he had demonstrated the disease in young larvæ, in older larvæ, in pupæ, in drones, in workers just gnawing out of the cell, in young nurse bees, in old, worn-out bees, and finally in the queen and eggs unlaid. Such data as are offered here by Cheshire, of course, are insufficient to prove any etiological relation of a bacterium to a disease.

CHESHIRE, SEPTEMBER 1, 1884.

In an earlier paper (p. 19) Cheshire adhered to the view held by Dzierzon and others, that foul brood was of two kinds.

The view which he expresses in a later article [1] is that there is but one disease and that one is caused by *Bacillus alvei*. After he had examined a number of samples of comb affected with the disease he reached the conclusion that Dzierzon was in error in asserting that there were two kinds of foul brood, one mild, the other malignant. While all cases of foul brood, he says, are due to *Bacillus alvei* and to this extent are identical, yet in some cases the spores of this organism are larger, more robust, and more virulent than in others. When the disease manifests itself early in the development of the brood he contends that it is more difficult to cure, if any difference exists, than when the symptoms appear later in the disease. He contends further that if this disorder is due to the disease lurking in the queen she must be removed.

The doubt that was entertained as to the effectiveness of the carbolized sirup treatment caused Cheshire to perform another experiment. In a healthy colony he placed, on August 6, six combs secured from more than one source, and all affected with foul brood. On the following morning he poured medicated sirup into the combs. Similar feedings were continued daily in liberal quantities until the sixth morning. After this a tin-pan feeder was used, which was not allowed to become empty of sirup. Eggs were laid and brood reared in the foul-brood combs which had been inserted. Almost all the larvæ that were reared were healthy. Many of those that were near the lower edge of these frames, however, were affected and passed through the first stages of the disease. These larvæ later disappeared by being carried out, it was supposed, by the workers. Only three or four sealed cells with diseased brood now remained, and it was believed that these would be cleaned out by the bees as soon as the cappings were broken. With these exceptions, he says, the hives were, on August 23, as perfect as could be desired. Following the description of his experiment, Cheshire writes:

[1] Cheshire, Frank R., September 1, 1884. The Cheshire treatment of *Bacillus alvei* (foul brood). British Bee Journal, Vol. XII, No. 153, pp. 294-296.

The Bee Keepers' Record says, in referring to my paper, "Whether phenol is really a specific for foul-brood time alone will show, but we urge our readers to give it a thorough trial." I reply that all that could be done to prevent phenol succeeding I have done. I have heaped up difficulties: given bees such combs as I venture to say they have never received before in the history of bee keeping; secured the most virulent type of the disease I could discover, and yet in seventeen days a most perfectly healthy aspect is presented, and the bees, with brood in their six frames, are hard at work comb-building. I assert, with all the positiveness I can command, that phenol, upon my plan, is a specific, and only needs a careful and correct application.

Bee keepers who have had experience in the treatment of foul brood can decide, first, whether such phenomenal results as Cheshire has recorded are to be expected, and, second, whether from one experiment like this he should have asserted so positively that the method is a specific one.

The method advocated by Cheshire was given a trial, however, by many bee keepers, especially in England. To indicate what harm might ensue from such immature work, we quote from a few who followed his advice. A questioner[1] gives the following from his experience:

Having used carbolic acid as a prophylactic in the apiary for more than fifteen years, I was delighted to learn that Mr. Cheshire, by putting a little amongst syrup and pouring it into the brood-cells of a virulently diseased stock, had succeeded in effecting a complete cure. To test its power in this way I procured a bottle of medicated material prepared under Mr. Cheshire's guarantee, and began to treat a stock, thoroughly foul-brooded, according to the method prescribed—on the 30th August last.

Circumstances were all favourable, such as a high temperature, a breeding queen, and bees carrying in pollen. No heat was allowed to escape from the stock through imperfect covering. But although the treatment has been carried on till now (Oct. 20th), there is no more abatement of disease than usually takes place when egg-laying becomes languid. The population is getting reduced; the cells perforated and closed are filled with gluey, putrid matter, and the stench emitted is scarcely less offensive than formerly.

What the treatment can effect in spring and summer, when greater heat and activity prevail, remains to be tested; but from what has come under my observation I have come to the conclusion that unless the apiarian himself clear out every foul cell, no virulently diseased hive can be restored to perfect health in the autumn by administering phenol as Mr. Cheshire directs.

From another bee keeper[2] we quote the following:

I have used Mr. Cheshire's cure, and followed his directions to the best of my ability, but it has proved in my case a complete failure.

From another[3] the following is quoted:

During the latter end of July I observed that three of my bar-frame hives were affected with foul brood. I was a little puzzled what to do, as I never had had any

[1] Questioner, November 1, 1884. Phenol no cure in autumn. British Bee Journal, Vol. XII, No. 157, p. 379.

[2] Johnston, Arthur B., November 1, 1884. Is phenol a cure for foul brood? British Bee Journal, Vol. XII, No. 157, p. 379.

[3] Veritas, November 15, 1884. Foul brood. British Bee Journal, Vol. XII, No. 158, pp. 399–400.

experience of nor had I seen the disease before. However, from the confident way in which Mr. Cheshire spoke of his phenol cure, I resolved to try it, and as honey was still coming in, I had to pour the medicated syrup over the brood-combs; but as soon as the ingathering of honey ceased, I extracted all the combs in my apiary and commenced to feed; and after a little experience of it, I found that 1 in 600 was as much as the bees would take. When enough of this had been deposited and sealed for winter stores, I made a thorough examination, and found that it had not cured a single one, but the disease had spread to others that were being fed with the carbolised syrup. I then withdrew the combs from two of the worst, and gave them empty ones to begin in, re-fed them 1 in 600 of Calvert's No. 1; the disease spread again, and I lost all faith in the Cheshire cure.

Cheshire, September 15, 1884.

Two weeks later another article[1] by Cheshire appeared in which the origin of the names *Bacillus depilis* and *Bacillus gaytoni* is found.

Many cases had been reported in which numerous small, hairless bees had been found in front of the hive. These had been considered simply as robbers. It seems, however, that Miss Gayton, a bee keeper and observer, had furnished Mr. Cheshire some of these bees, together with her notes for examination. He examined the bees and found in them in every case a bacillus smaller than *Bacillus alvei*, and by work done in the Biological Laboratory at South Kensington they were believed to be entirely distinct species. In his paper Cheshire writes:

> This bacillus, undoubtedly, produces this effect [premature baldness] and so again I claim the right of giving a name, and so suggest *Bacillus depilis*, or the bacillus of hairlessness, as a fitting one. Although, perhaps, *Bacillus gaytoni* would be better remembered and only a well-deserved compliment.

Here again it is noted that data are wanting to justify the conclusions drawn.

Cheshire in the same article gives also a few laboratory notes of some interest to support his view that *Bacillus alvei* is the cause of foul brood. He, together with Cheyne, inoculated some gelatin tubes with a small quantity of the coffee-colored remains of diseased cells. Subcultures to the seventh generation were made. The character of the growth thus obtained indicated that the organism was unknown. The cultures were described as having the same characteristic odor that is encountered in hives containing foul-brood material. This he believed to be strong evidence that *Bacillus alvei* is the cause of foul brood. In this same paper he anticipated the proof to be obtained from the inoculation of a healthy colony with *Bacillus alvei*. The twelfth generation of the culture was to be mixed with water and sprayed over a card of healthy brood. Concerning the results to be obtained he writes: "I will not prophesy, although I foresee the results." At that stage of his investigation it was unwise, of course,

[1] Cheshire, Frank R., September 15, 1884. Bee diseases in relation to apiculture and general science—*Bacillus gaytoni* (?). British Bee Journal, Vol. XII, No. 154, pp. 317-318.

to entertain such hopes. It might at first seem to the reader that Cheyne was jointly responsible with Cheshire in these statements, but it will be learned later that the responsibility is with the latter.

Cheshire, October 15, 1884.

In another paper[1] which appeared one month later, Cheshire considers the possibility of the transmission of foul brood from drones at the time of mating. He received from a bee keeper a queen that had accompanied a swarm, and after beginning to lay for the new colony she ceased after about 6 square inches of comb had been filled and never laid again. Upon post-mortem examination the ovaries and spermatheca were apparently normal to the naked eye. The contents of the spermatheca were examined under the microscope and many bacilli were found among the spermatozoa. An inflamed condition of the mucous gland and valves was reported to be present, and this fact was given as the probable reason why the oviposition had been arrested. The theory advanced by Cheshire to account for the disease was that the colony had recently cast a swarm unobserved, and that the queen which had been sent him for examination was a young one and in mating had contracted from the drone the condition that was observed microscopically. This supposition seemed probable to him, because he had seen what he identified as being *Bacillus alvei* among the spermatozoa of drones taken from foul-brood colonies.

Cheshire cites another case: A queen had been sent to him with the information that she was an old one. Upon examination he concluded, on the contrary, that she was young and badly diseased, since among the spermatozoa, as in the first case, many bacilli were observed. Naturally from such observations Cheshire did not prove that the cause of foul brood could be transmitted from drone to queen at time of mating.

Cheshire and Cheyne, August, 1885.

The paper which was prepared by Cheshire and Cheyne[2] conjointly, as the result of the work mentioned in this quotation, was read on March 11, 1885, and was published in August, 1885. It appears in two parts. Part I, written by Cheshire, considers the pathogenic history of *"Bacillus alvei,"* and Part II deals with the history of *Bacillus alvei* under cultivation, which is the portion written by Cheyne. The part written by Cheshire contains a sum-

[1] Cheshire, Frank R., October 15, 1884. A new discovery with regard to bacillus disease. Diseased spermatheca. British Bee Journal, Vol. XII, No. 156, pp. 355-356.

[2] Cheshire, Frank R., F. R. M. S., F. L. S., and Cheyne, W. Watson, M. B., F. R. C. S., August, 1885. The pathogenic history and history under cultivation of a new bacillus (*B. alvei*), the cause of a disease of the hive bee hitherto known as foul brood. Journal of the Royal Microscopical Society, Ser. II, Vol. V, Part 2, Plates X and XI, pp. 581-601.

mary of his work on foul brood which appeared for the most part in the papers by him which we have already reviewed. In addition to the work contained in his former papers he reports the results of his experimental inoculation of healthy larvæ with cultures of *Bacillus alvei.*

On page 24 of this bulletin it will be noted that Cheshire outlined briefly the manner in which the inoculations would be made, and stated furthermore that he could foresee the results. After obtaining the results of his experimental inoculation, he writes as follows:

> It is needful before passing to the second head to anticipate one or two points to which Mr. Watson Cheyne will especially refer. After very many cultivations conducted in series by that gentleman, a small quantity of sterilized milk was inoculated from the last tube. It behaved characteristically, as Mr. Cheyne will describe, the flask emitting upon the drawing of the plug the unmistakable odour so distinctive of the disease in the hive. Some of this milk I diffused through water and sprayed from an atomizer over a healthy comb of larvæ, part of which was protected by a cardboard sheet into which four lozenge shapes had been cut. The larvæ protected matured in health; those exposed to the spray in many cases were removed by the bees, while the rest died, their bodies filled with *Bacillus alvei.* This last experiment seems to complete the chain of evidence in favour of "foul brood" not being accidentally associated with this bacillus, but actually its result.

If positive results can be obtained by experimental inoculation of course such results furnish evidence which is of the greatest value in the determination of the cause of the disease. Cheshire's experiment, however, did not furnish such evidence. It will be noted that Cheshire states that some of the larvæ died with their bodies filled with *Bacillus alvei,* and while he does not say positively that the larvæ died of foul brood, this idea is likely to be inferred by the next statement that is made. While these statements by Cheshire are made with some degree of conservatism, they evidently have been interpreted by many to mean that the disease was produced, and this conception has led to a great deal of confusion in the minds of bee keepers concerning the cause of foul brood. Cheshire states that Cheyne will especially refer to the experimental inoculation of healthy brood which he only anticipates in his part of the paper. In considering Cheyne's contribution it will be seen in what way he disposes of this very important phase of the investigation.

This completes the consideration of the papers by Cheshire as far as we purpose to deal with them at present. Before taking up the investigations of Cheyne on *Bacillus alvei* it may be well to summarize Cheshire's papers on foul brood.

1. Having accepted an invitation from the British Bee Keepers' Association to give an address upon foul brood, Cheshire began to study the disease about the last of May, 1884, although he mentions having examined, microscopically, larvæ dead of the disease some years before.

2. He gave the results of the first two months' work before a conference of this association on July 25 of the same year.

3. Although he started out with Dzierzon's idea that two forms of foul brood are to be encountered in studying the disease in the apiary, he does not seem to have suspected, while making his observations, that probably two distinct diseases were being called by the one name—foul brood.

4. The observations which he made on the morphology of the bacillus found in the diseased and dead larvæ were very good. He probably saw what we now know as *Bacillus larvæ*, but interpreted his findings wrongly. Before Cheshire and after him there were several who probably encountered the same bacillus in their studies, but who made the mistake of misinterpreting their results. Inasmuch as American foul brood is widely distributed in many countries, and *Bacillus larvæ* is always found in the larvæ dead of the disease, it would have been almost impossible for these men not to have seen this microorganism.

5. Cheshire, by his studies on the morphology of the bacillus, by his inoculation experiments on blowfly larvæ, and by cultures, attempted to prove that Schönfeld was in error in his investigations. It is true Schönfeld had not proved his theory concerning the etiology of foul brood, but Cheshire in his attempt to do so failed to prove that Schönfeld was in error.

6. Cheshire began the study of *B. alvei* culturally with a medium prepared from the larvæ of bees. He was unfamiliar, however, with the technique used in cultural methods, and for this reason too much importance should not be attached to his results. Historically, however, it is of interest, since it was probably the first cultural work to be done in the study of bee diseases.

7. Inasmuch as he inoculated healthy brood with cultures, one learns that Cheshire recognized the advisability of making animal inoculations in determining the cause of disease. Unfortunately here, too, the methods he used were deficient, and his interpretation of the results obtained misled many as to the cause of foul brood.

8. Cheshire had suspected from some observations which he had made that adult bees suffered from foul brood. Examining microscopically the content of the intestinal tract of an adult bee taken from a foul-brood colony, he found many active bacilli to be present, and from this observation he was convinced that adult bees, as well as larvæ, suffer from the disease. Had he, however, examined a healthy bee in the same way, he would probably have seen a similar condition.

9. He gave the name *Bacillus alvei* to the bacillus with which he was working. "*Alvei*," used to designate the species, is very similar to the word "*alvearis*," which Preuss used (p. 16) to designate the

species of the microorganism, *Cryptocöccus alvearis*, seen by him in foul-brood larvæ. The two may have been the same germ.

10. From his own work there is no way of knowing positively with what bacillus Cheshire was working, since he made no satisfactory description for its identification. Later (pp. 31–33) Cheyne made a careful description of *Bacillus alvei*, and Cheshire agreed that it was the organism to which he had given this name.

11. Cheshire asserted positively that his phenol treatment is a most effective one for foul brood. Many bee keepers have tried it, however, without success.

12. He concluded upon further study that the two forms of the disease described by Dzierzon are one disease, and that this disease is amenable to the "Cheshire treatment," even when the disease appears in the most malignant form.

13. Concerning the difference noticed in samples examined microscopically, he writes that the more robust spores are associated with the more virulent disease.

14. He was led to believe that the "premature baldness" of "black robbers" was due to a bacillus which he saw and named *Bacillus depilis* or *Bacillus gaytoni*.

15. He reported that the odor of a gelatin culture of what he supposed was *Bacillus alvei* was very similar to the odor observed in colonies affected with foul brood. Even if *Bacillus alvei* were the cause of a disease of the brood, one should not expect, of course, this similarity.

16. He suggests the possibility that a queen at the time of mating might become infected with *Bacillus alvei* from a drone reared in a foul-brood colony. He expressed a strong conviction that in this way foul brood might be transmitted to a healthy colony.

17. He would have his readers believe that he had found the disease in young larvæ, in those fully fed, in chrysalids of all stages, in drones, in workers just gnawing out of the cell, in young nurse bees, in old worn-out bees, and in the queen and the unlaid eggs.

18. All of Cheshire's papers which have been considered—and we have not referred to them all—were prepared in less than one year and most of his observations were made in less than half that time.

We have reviewed these papers by Cheshire in order to point out the origin of some of the errors that have crept into bee literature. That the several suggestions made by Cheshire were never demonstrated to be true will at once be apparent to the reader. The following criticism offered by Cheshire[1] on the work of Schönfeld may now be applied, it seems, with equal propriety to his own:

> I cannot refrain from expressing my conviction that it is much to be regretted that so misleading an account of experiments, to all appearances conclusive and complete,

[1] Cheshire, Frank R., August 1, 1884. Foul brood (not Micrococcus, but Bacillus), the means of its propagation and the methods of its cure. British Bee Journal, Vol. XII, No. 151, pp. 256–263.

should have been given to the apicultural world. In their absence, it is hardly possible that we could have all been in the dark so long.

Because of the important bearing which the work of Cheshire and Cheyne has upon the names of the two infectious bee diseases, and upon the names now applied to the bacteria found in the diseased larvæ, it might be well before taking up the work of Cheyne to consider these two men briefly and judge from the evidence at hand their relation to each other.

Cheshire was a man who wrote considerably upon bees and bee keeping, being apparently more or less familiar with the habits, anatomy, and manipulation of bees. From his writings about the diseases of bees, however, one at once suspects that his experience in this line of apiculture was quite limited. His conception of the etiology of diseases in general was evidently very inaccurate. His bacteriological knowledge was wanting. Of this he was undoubtedly aware, as he later intrusted this part of the work to Cheyne, who was then working in a biological laboratory at South Kensington, London.

Cheyne is a man who is familiar with the technique of disease investigation. He was apparently, however, not familiar with the disease, foul brood, at the time he received the sample from Cheshire. This fact, however, does not discredit the actual work which Cheyne did.

If this was the relation existing at that time between these two men, and if this is a correct interpretation of their knowledge, respectively, of foul brood, then it is not strange that Cheyne should have been slightly misled by the opinion of Cheshire on two very important points in his work. These two erroneous ideas which Cheyne evidently gleaned from Cheshire were, firstly, that foul brood was one disease, and secondly, that the disease had been produced by Cheshire experimentally by using pure cultures of *Bacillus alvei*.

Cheyne, August, 1885.

Cheyne in his contribution[1] records the first creditable work done on the microorganisms found in foul brood. One observes that Cheshire writes:[2]

To-day [August 11, 1884] I have been with Mr. Watson Cheyne in the Biological Laboratory, South Kensington, and there we have started some experiments, of which more will have to be said hereafter * * *.

And Cheyne begins his paper by writing:

On August 11, 1884, Mr. Cheshire brought to me a piece of comb containing larvæ affected with foul brood * * *.

[1] Cheshire, Frank R., F. R. M. S., F. L. S., and W. Watson Cheyne, M. B., F. R. C. S., August, 1885. The pathogenic history and history under cultivation of a new bacillus (*B. alvei*), the cause of a disease of the hive bee hitherto known as foul brood. Journal of the Royal Microscopical Society, Ser. II, Vol. V, Part 2, Plates X and XI, pp. 581-601. (For the portion written by Cheyne see also Report of the meeting of inspectors of apiaries, San Antonio, Tex., November 12, 1906. U. S. Department of Agriculture, Bureau of Entomology, Bulletin No. 70, June 17, 1907, pp. 28-35.)

[2] Cheshire, Frank R., August 15, 1884. *l. c.*

The sample of comb which Cheshire gave to Cheyne for examination then was considered by Cheshire to be foul brood, and therefore was thought by the latter to be a sample of the only form of the disease that exists under this name. Further Cheyne writes, "selecting cells which were closed, but which Mr. Cheshire thought contained diseased larvæ, * * *." This statement indicates that Cheyne was unfamiliar with the gross appearance of the disease as it is found in the combs. After uncapping the cells containing foul-brood larvæ, he further writes, "these larvæ were dead, of a yellowish colour, and almost liquid, * * *." Capped cells occur more often in American foul brood, but are not rare in European foul brood. The yellowish color and the almost liquid condition are symptoms which would rather strongly suggest that the sample which he examined was European foul brood. Numerous rods were found microscopically in the diseased larvæ. Cultures were made in gelatin and in agar. The bacteria found in the larvæ and those which appeared in the cultures by comparison seemed to be the same. The morphology and cultural characters of this bacillus (*Bacillus alvei*) were carefully studied.

Concerning the method by which multiplication takes place, Cheyne writes:

> The bacilli appear to grow mainly by fission, but I have seen appearances which seem to me only explicable on the supposition that they also grow by sending out buds from one end.

A study of the germination of the spores was made, using the hanging-drop preparation. A drop of bouillon was placed on a cover-glass, inoculated with the spores of *Bacillus alvei*, and inverted over a cell in a glass slide. Preparations made in this manner were placed at a temperature favorable for the growth of the bacteria, and from time to time studies were made of them by the aid of the microscope. He writes:

> In three hours the first indication of sprouting of these spores becomes evident. The stained part of the spore loses its oval shape, becomes elongated, and is soon seen to burst through the spore-capsule at one part.

From this it is not possible to know whether he observed the capsule to burst on the side or at the pole, but the figure to which he refers shows the rod bursting through the capsule at or near the pole.

Having studied the germination of the spores, he proceeded to study the formation of the spores in the rod. In doing this two methods were employed. The first was by use of the hanging drop, similar to that used in his study of the germination. By this method he observed in one preparation that most of the rods were beginning to form spores in 23 hours, while in another preparation where more bouillon was used no evidence of spore formation was present during

the first 28 hours. This led him to conclude that the time when spores are produced might depend upon the amount of bouillon used and the number of bacilli present. This caused him to devise a second method. The second method involved the use of a flask of sterile bouillon, which was inoculated with the vegetative form only of the bacillus. This flask was placed in the incubator for two or three hours that the organisms might diffuse equally throughout. The flask was then shaken, and by means of a syringe gauged on the piston equal quantities of the medium and bacilli were taken. In a series of preparations thus obtained and kept at 36° C. the earliest appearance of spore formation was evident in 41 hours.

Cheyne records the fact that the swelling in the rod which takes place is usually near the center, but sometimes nearer an end. This swelling increases in size at its center and gradually fails to take the stain. The capsule of the spore, he states, is apparently formed within the rod and is not the outer part of the rod. In three or four hours after the spore was formed it was either entirely free from the rod, or the rod still inclosed the spore, but was almost invisible.

Having thus studied somewhat carefully the morphology (size, form, and structure) of *Bacillus alvei*, Cheyne took up the further study of the species to determine its cultural characters, stating at this point, very properly, that the microscope is of little use in determining the relation of the bacillus to foul brood. The technique which he used in making cultures is very similar to that used in general bacteriological work at the present time, the chief difference being that now additional differential media are used.

DESCRIPTION OF BACILLUS ALVEI.

The following is an abridged description of *Bacillus alvei* as given by Cheyne:

Occurrence.—Isolated from larvæ said by Cheshire to be affected with foul brood. As far as is at present known it has not been found elsewhere.

Gelatin plates.—Gelatin plates were inoculated by stroking the solidified medium with the needle. From small masses of growth, which soon form along this line of inoculation, bacilli in Indian file, or two or three side by side, grow out into the gelatin. These outgrowths are not straight, but tend to curve, and at a short distance from the track of the needle they grow round so as to form a circle. From such a circle other fresh circles may be formed. The growth about this line increases, filling up the center of the circle. These circular growths increase and may join one another, forming a curved anastomosis. The gelatin in the immediate vicinity of the growth

liquefies, and in these liquid channels the bacilli swim to and fro. Later some of these channels are apparently deserted of bacilli, so that the circles may look as though they were detached from the main track. Under low powers, however, the connection between them can be traced.

If the gelatin is inoculated before pouring the plate, the growth which takes place is also very characteristic. At first the colonies are small and oval or round. Under a low power of the microscope the colony does not appear homogeneous, but lines indicating the bacilli are seen. The colony soon becomes pear-shaped, and processes grow out from the sharp end of the pear into the gelatin.

Morphology: Rods.—In the larval juices they are rounded or slightly tapering at the ends, and often have a clear space near one end. Their average length is about $3\frac{1}{2}$ μ. On agar the rods are always somewhat pointed at the ends and varying in size. The average length is about $3\frac{1}{2}$ μ and the breadth about $\frac{5}{8}$ μ. At the beginning of spore formation the rods begin to swell and become spindle shaped. This increase in diameter is generally near the middle, but sometimes it is seen near one end. The capsule of the spore is apparently formed within the rod, and is not merely the outer portion of it. *Spores.*—The spores are oval, averaging nearly 2 μ in length and nearly 1 μ in breadth. Spores from agar cultures are generally arranged side by side in long rows.

Motility.—The bacilli swim with a free oscillating movement.

Bouillon.—Growth takes place readily, causing a cloudiness of the medium and the formation after a few days of a slight but not tenacious pellicle. The odor is similar to that described under gelatin. This character is more marked when considerable peptone is present. Probably there is no change in the chemical reaction.

Agar.—The growth is not nearly so characteristic as in gelatin. It takes place most rapidly on the surface, forming a whitish layer. Here the bacilli arrange themselves side by side and, forming spores in this position, there are after a few days, as a consequence, long rows of spores lying side by side.

Blood serum.—Growth takes place slowly, forming long filaments and comparatively few spores.

Potato.—At the incubator temperature the growth takes place slowly, forming a dryish yellow layer on the surface. It is very slow at lower temperatures.

Milk.—Growth takes place readily and coagulation occurs. The medium assumes a yellowish color and gives off the odor present in gelatin-tube cultures. The coagulation is not firm, but like tremulous jelly and may remain so for a considerable time before the sep-

aration of any fluid takes place. Ultimately, however, it becomes liquid, and after some months it assumes the appearance of a dirty brownish yellow, glassy fluid. It is very slightly, if at all, acid.

Gelatin tube.—In this medium the growth is seen on the surface and along the line of inoculation. On the surface a ramifying growth takes place from the point of entrance of the needle; and along the line of inoculation whitish, irregularly shaped masses appear, which increase slowly in size. In a few days processes, which are thickened at various points and clubbed at the ends, shoot out from these masses. The ends of these processes do not seem to unite. A beautiful appearance is obtained when only a few bacteria are introduced along the line of inoculation. In a few days small round colonies become visible to the naked eye. These increase in size, and at about the tenth day shoots begin to appear, which radiate in all directions from the central mass and become clubbed. As the culture becomes older the radiating branches disappear, and only small whitish collections of bacilli are seen at various points. Under slight magnification, however, tracks of liquefied gelatin are seen extending from the central mass to the whitish collections. The evaporation which takes place at the top gives rise to the appearance of an air bubble.

Beginning at the top the liquefaction of the gelatin advances slowly downward until ultimately the entire tube is liquefied. After two or three weeks there is a layer of liquid at the upper part, with the growth as before described in the lower part. At first the liquefied gelatin is clear excepting a loose, white, flocculent sediment which is present. There may be a thin surface pellicle. Later the liquid becomes yellow, and gives off an odor similar to stale but not ammoniacal urine. This, Cheyne says, may better be described as a "shrimpy" smell. "The yellowish color and the peculiar odor," Cheyne writes, "has been found by Cheshire to be distinctive of diseased larvæ."

Cheyne made also a few observations with animals inoculated with *Bacillus alvei*. A bluebottle fly which had eaten some of a milk culture of *Bacillus alvei* was placed under a funnel; the next day it was found dead, and upon examination its juices were reported to be full of the bacilli. Cockroaches were placed in a box with cultures, but none of them died. A rabbit, some mice, and some guinea pigs received cultures of *Bacillus alvei* subcutaneously, but in general only negative results were obtained.

A conclusion which Cheyne drew indicates that at that time he was pretty thoroughly convinced that *Bacillus alvei* was the cause of foul brood. The belief which he entertained was based upon his

study of *Bacillus alvei*, together with the experiment by Cheshire in which healthy brood was inoculated with a spray containing a culture of *Bacillus alvei*. In drawing the conclusion Cheyne evidently supposed that Cheshire had produced the disease experimentally. Had Cheyne known, on the other hand, that this had not been done by Cheshire, his conclusion would have been undoubtedly differently expressed.

The description which Cheyne made of *Bacillus alvei* is very good. It contains, however, a number of statements with which he, himself, no doubt at the present time would disagree. Such might be expected since it has now been 26 years since he did the work.

There is much data in Cheyne's paper of interest and value. The following is a brief summary of his work:

1. He received a sample of diseased brood from Cheshire on August 11, 1884, and the paper which contained the results of his work was read on March 11, 1885.

2. He described carefully and accurately the morphology and cultural characteristics of *Bacillus alvei*. His description of the organism is the first one by which the identification of the species was made possible.

3. The larvæ which he examined were yellowish and almost liquid. This suggests European foul brood.

4. He found *Bacillus alvei* in large numbers in all the larvæ examined. This, too, suggests European foul brood.

5. He does not mention either the presence of ropy coffee-colored larvæ or scales in the sample examined. This suggests that he was not studying American foul brood.

6. He evidently did not encounter *Bacillus larvæ*, since he does not mention the presence of any bacteria in the larvæ which would not grow on artificial media. This, too, is very strong evidence that he was not studying American foul brood.

7. He was misled by Cheshire's inoculation experiment with bees, causing a statement to be made in his conclusion which was less conservatively expressed than it would otherwise have been.

While Cheshire and Cheyne did not prove the cause of any disease of bees, their work is of importance in determining to what species of bacteria the name *Bacillus alvei* belongs, and also in determining the names for the different brood diseases. The microorganism which Cheyne so well described has the right to the name *Bacillus alvei*, because the species was first described by Cheyne and his work had the sanction of Cheshire, who first used the name.

It is quite certain that Cheyne was working with the disease now known as European foul brood when he secured the data for his

paper, because his description of the larvæ dead of the disease in the sample which Cheshire gave to him as foul brood was quite good for European foul brood and not good for American foul brood; because *Bacillus alvei* was found in sufficiently large numbers to lead these men to suspect that the organism was the cause of the disorder; and because no other species was mentioned as being present in numbers sufficient to cause suspicion of a casual relation. All must agree that if Cheyne did not work with a sample of European foul brood in the preparation of his paper, his work can not be given the credit which it seems to deserve.

McLAIN, 1887.

A paper by N. W. McLain,[1] containing a discussion of bee diseases, was written in the form of a report on some work done by him. The first disorder which he considers is referred to as the "quaking disease." It was thought by McLain that bees would visit milkweed and mullein to obtain from the sap of these plants certain salts as food, if such salts could not be obtained from the ordinary sources. In so doing, thousands of bees lost their lives before, as well as after, reaching the hives. By examining such bees under a microscope, many were found to be entangled in filaments derived from the sap of the plants visited, and with empty stomachs. The peculiar nervous motions made by these starved and weakened bees in their effort to disentangle themselves from the meshes of the fibers is one manifestation of the condition known as the "quaking disease." Another form of this disease was supposed to be of hereditary origin, since it was believed that by removing the queen from an affected colony and introducing a young, vigorous one the trouble would disappear. A third form of the disease mentioned had been reported to be due to the use of poisonous nectar from such plants as foxglove (Digitalis).

McLain therefore placed at least three distinct abnormal conditions under the name "quaking disease." He writes definitely concerning the cause of the first condition only. The second condition is probably that which is now known as paralysis, and the third condition, that of poisoning, is occasionally reported by bee keepers as a cause of trouble in the apiary. Very little is definitely known at present concerning any of these disorders. In the treatment of the first condition mentioned he used a drug and reported success.

McLain also made a report on foul brood. Having spoken of the gravity of the disease he writes that he had during the year given much attention to the study of the disease and to experiments for its prevention and cure. That the disease was contagious appeared to

[1] McLain, N. W., 1887. Report on experiments in apiculture. Report of the Commissioner of Agriculture for 1886, pp. 583–591. Washington: Government Printing Office.

him to be certain. The origin and the means of its transmission, however, were not entirely clear. That the germs of the disease may be carried from apiary to apiary upon the clothing of the apiarist and in or upon the bodies of bees, that in the same apiary these germs may be borne by the winds from one hive to another, and that they may be liberated from the decomposing bodies of other insects and scattered to objects with which the bees come in contact, seemed to McLain to be probable. It appeared to him that foul brood attacked adult bees as well as brood, that live pollen is the medium by which the contagion is most commonly and most rapidly spread, and that the disease yields readily to a drug treatment.

In discussing the idea that the clothing and hands of an individual going from one apiary to another might probably be a means by which disease germs are transmitted, he writes:

> That the disease germs may be carried upon the clothing and hands appears probable, from the fact that in one neighborhood the disease appeared in only two apiaries, the owners of which had spent some time working among diseased colonies at some distance from home, while other apiarists in that locality who had kept away from the contagion had no trouble from foul brood.

In support of his supposition that the wind might be considered as a medium by which the germs of the disease may be carried, he writes:

> That the contagion may sometimes be borne from hive to hive by the wind appears to be true, as it was observed in one of the apiaries which I treated for this disease during the past summer that of a large number of diseased colonies in the apiary, with the exception of two colonies, all were located to the northeast of the colony in which the disease first appeared. The prevailing wind had been from the southwest.

The report covers the work done by McLain in one year on bee diseases. He was conducting at the same time some experiments relative to the control of the mating of the queen. Most of his conclusions concerning diseases were drawn, apparently, from three experiments performed by an apiarist who reported his results to McLain.

The following is a summary of his report pertaining to bee diseases:

1. He made no pretense at a study of bee diseases bacteriologically.
2. He included at least three distinct conditions in the disease of adult bees which he referred to as "quaking disease."
3. He probably included in the "quaking disease" the disorder which is now known to many bee keepers as "paralysis."
4. He recognized the virulence, wide distribution, and, consequently, the destructiveness of foul brood.
5. He probably was not aware that at least two infectious diseases of bees were being referred to as foul brood.
6. Since American foul brood has been the prevailing disease in the region in which his experiments were made, and since the descrip-

tion which McLain gives of the appearance of the dead larvæ is that of American foul brood, he very probably was working with this disease.

7. He seemed to accept the belief that adult bees are affected with foul brood as well as the brood.

8. He supposed that pollen is the medium by which the infection is usually transmitted.

9. He evidently believed in the efficiency of the drug treatment of bee diseases.

Let us now consider for a moment some of his contentions. One must agree with McLain that the diseased condition then known as foul brood is an exceedingly serious one, causing great loss to the bee keepers. That the disease is quite infectious has often been demonstrated. That the germs of the disease may be carried from one hive to another in and upon the bodies of the bees seems very probable. That the germs are carried from one colony to another upon the clothing of the bee keepers and that the infection is transmitted in this way is extremely improbable. That the germs are carried by the winds from one hive to another in the apiary is likewise very improbable. That the germs of the disease are liberated from other insects and afterwards taken up by bees is not probable.

In many ways this report by McLain is a conservative one. Sufficient evidence was wanting to prove most of the points in his paper. He probably realized this fact, and for this reason, as a rule, he did not make positive statements. Inasmuch as his report covers the work of a single season, very little definite information could be expected.

McLAIN, 1888.

In his report [1] on the succeeding year's work McLain discusses somewhat again the question of bee diseases. This report shows the influence of Cheshire's writings, since McLain now speaks of the inappropriateness of the name foul brood (p. 21) and the certainty of the etiological relation between *Bacillus alvei* and the disease (p. 20). Furthermore he refers to the statements of Cheshire concerning the probable spread of disease through the air and by means of the clothes and hands of the operator, and says that Cheshire's observations are in agreement with his own which he included in the preceding report.

McLain had expressed (p. 36) his firm belief in the theory that pollen was the common source of infection in foul brood, and not honey, as was commonly supposed. This view, he thought, was strengthened by some statements which Cheshire made. McLain was of the opinion that undue importance was being attached to honey as a medium through which the infection is transmitted, and

[1] McLain, N. W., 1888. Report on experiments in apiculture. Report of the Commissioner of Agriculture for 1887, pp. 170–178. Washington: Government Printing Office.

expressed his convictions that there was but little doubt that pollen is the medium by which the contagion is most commonly introduced, most rapidly spread, and most persistently perpetuated.

McLain also includes in his report some remarks on starved brood, and in referring to the symptoms states that in this condition the brood is frequently found to be only partially capped, giving the appearance commonly designated by the term "baldheaded brood."

In estimating the value of this work by McLain, it must be borne in mind that McLain had evidently a very indefinite conception of the phenomena which are encountered and must be dealt with in the study of disease; that he devoted but little time to the study of the disease he referred to as foul brood, and that he was probably unduly influenced by the writings of Cheshire. For these reasons it is advised that his reports be not taken too seriously.

Lortet, February, 1890.

A paper[1] by Dr. Lortet concerning some of the bacteria encountered in the study of foul brood appeared in 1890. He found two species always present in the digestive tract of healthy adult bees as well as in those diseased. These species were reported to be present also in the digestive tract of both healthy and diseased brood. The fact was pointed out that these bacteria had probably led some authors into error in their work. The two species were not named nor sufficiently described to make their identification possible. He encountered also, in the digestive tube of brood diseased and dead of foul brood, another species which he supposed was *Bacillus alvei* and which was the cause of the rapid death of the larvæ. Lortet records no difficulty in cultivating this species on the ordinary media.

It was his belief that adult bees suffered from the disease, but that they resisted the infection more than the larvæ, and finally died as a result of the infection. Experimentally, he claims to have obtained positive results in support of his views. He examined one queen taken from an infected colony and from a study of her he reported that she was perfectly healthy and that her eggs were free from bacteria. It was his opinion that food was the source of infection of the digestive tube of the nurse bees and that the nurse bees became in turn the source of infection for the brood.

From his work he drew the following three conclusions: (1) That Cheshire had found the true exciting cause of foul brood and declared that the fact had been verified by numerous laboratory experiments; (2) that it is useless to attempt to save larvæ already infected, and (3) that adult bees which become infected may live a long time, and some may even resist the attack completely.

[1] Lortet, Dr., February, 1890. La bacterie loqueuse. Traitement de la loque par le naphtol β. Revue Internationale d'Apiculture, Tome XII, No. 2, pp. 50-54.

Believing that the digestive tract of the adult bee was the source of infection for the larvæ, he recommended, as the most rational treatment, the use of an intestinal antiseptic in the form of sirup medicated with beta naphthol, a drug which had been used for some time as an intestinal antiseptic in the practice of human medicine. The feeding of sirup medicated with beta naphthol (one-third gram beta naphthol to 1,000 grams of sirup), he reports, was sufficient in his experiment to free the intestinal tube of the bacteria causing the trouble. This cure he supposed took place rapidly and completely except when the bacteria had reached more completely the different portions of the alimentary tract.

One observes that the views entertained by Lortet on bee diseases are quite different from those entertained at the present day concerning these disorders.

MACKENZIE, DECEMBER, 1892.

In 1892 Mackenzie read a paper[1] on *Bacillus alvei* which he had prepared at the request of the Bee Keepers' Union of Canada. The relation which was supposed by Mackenzie to exist between foul brood and *Bacillus alvei* is shown in the following brief review of his paper:

He received from a bee keeper some samples of diseased brood for examination. He began the study of these samples upon the assumption that Cheshire and Cheyne had already found *Bacillus alvei* and by inoculation with pure cultures had demonstrated this organism to be the cause of the disorder. By finding an organism which he thought from its morphology and cultural characters was *Bacillus alvei*, the conclusion was reached that the samples were foul brood, the same as was found in other places.

Laboring under the erroneous conception that *Bacillus alvei* is the cause of the foul brood prevalent in Ontario, Mackenzie proceeded with the study of the bacillus identified by him as *Bacillus alvei*. The first task mentioned in his paper which was undertaken by him was the solution of the question whether in the making of wax foundation sufficient heat is applied to destroy the vitality of the foulbrood spores. After receiving replies from different foundation manufacturers concerning the highest temperature reached in the process and the time the wax was kept at this temperature, and after making some determinations of the thermal death point of the spores of the bacillus, he writes: "I am inclined to think there is little danger from foul brood in that direction." He found by a cultural method

[1] Mackenzie, J. J., B. A., December, 1892. The foul brood bacillus (*B. alvei*); its vitality and development. Eighteenth Annual Report of the Ontario Agricultural College and Experimental Farm, pp. 267-273.

This address is quoted in full in the Report of the Meeting of Inspectors of Apiaries, San Antonio, Tex., Nov. 12, 1906. (Bulletin No. 70, Bureau of Entomology, U. S. Department of Agriculture, 1907, pp. 36-42.)

that 2 per cent carbolic acid would not kill the spores of *Bacillus alvei* in six hours, and concluded that carbolic acid in this strength could not be relied upon as a hive disinfectant to destroy the spores of this organism.

Mackenzie says that if the shaking treatment is employed, the question of the presence of *Bacillus alvei* in the workers, queen, and eggs must be considered in the discussion of the value of such treatment. He claims to have confirmed the results obtained by Cheshire that the bacilli are sometimes found in the intestine of the worker and the ovaries of the queen, but that the finding of the bacillus in the eggs of an infected queen needs confirmation.

He reports an experiment from which he concluded that carbolic acid (1–500), as used in medicated sirup in the treatment of foul brood, does not kill the spores but prevents their germination, and thus gives the bees a chance to rid themselves of the infection. "Its advantage," he writes, in comparing it with a shaking method, "is that it can be carried on for a longer time." Concerning beta naphthol, he concludes that a 1–1,000 solution in bouillon possesses the same value as an antiseptic as a 1–500 solution of carbolic acid and he believed that it would probably be more acceptable to the bees. The use of salicylic acid was thought by him to be followed by about the same results as carbolic acid and beta naphthol in the medication of sirup. For the cleaning of hives and frames he recommends a 10 per cent solution of soft soap or washing powder.

The following points of interest are found in Mackenzie's paper:

1. He accepted the work of Cheshire and Cheyne as demonstrating conclusively that *Bacillus alvei* is the cause of foul brood, and used in his laboratory experiments a bacillus which he identified as this organism.

2. At the time Mackenzie's paper was written his work on foul brood had been carried on for only about a year, and he appreciated the fact that his work was by no means complete.

3. From the report of foundation manufacturers and from the results of his own investigations, he was inclined to believe that there is but little danger of infection from foundation made from wax taken from a foul-brood colony.

4. He isolated *Bacillus alvei* from the ovaries of three of the five queens examined, but believed that the findings of Cheshire with respect to the eggs need confirmation.

5. He interpreted the finding of *Bacillus alvei* in the intestines of workers and queen as a fact worthy of consideration in the shaking treatment.

6. He believed that drugs in the treatment of foul brood have a value in preventing the germination of the spores.

The greatest mistake made by Mackenzie in his work was of course that he assumed that *Bacillus alvei* had been demonstrated to be the cause of foul brood.

The work which Mackenzie began was to have been continued the following summer, but, in a report [1] of the Apicultural Committee of the Ontario Agricultural and Experimental Union one notes that Mackenzie, who had the preceding year given his services in connection with foul brood, for the want of time had not continued his studies.

Howard, March 1, 1894.

In 1894 there appeared from the pen of William R. Howard,[2] of Fort Worth, Tex., a small publication on foul brood. He makes clear in his preface that there is yet much to be learned upon the subject, and that his communication is to be written in such a manner, and such terms are to be used in it, as will be readily understood by the general reader.

In his brief reference to the history of foul brood, Howard writes:

> Later the researches of Preuss and Schönfeld, of Germany, were first to establish the fact that the disease was due to pathogenic micro-organisms.

Howard, therefore, in the beginning entertained an erroneous conception concerning the real work accomplished by Preuss (p. 15) and Schönfeld (p. 16).

The description given of *Bacillus alvei* was taken, as he says, from "Eisenburg's Bacteriological Diagnosis." The description of *Bacillus alvei* by Eisenburg was compiled from the joint publication by Cheshire and Cheyne (p. 25). Howard made some determinations concerning the ability of the species to produce gas and the ease with which it grows in the presence or absence of oxygen. He reports that the cultures, when grown under anærobic condition, produce an odor resembling foul brood. Eisenburg does not include in his description any mention as to the oxygen requirements of *Bacillus alvei*. The only difference, it seems, between the description which Cheshire and Cheyne made of *Bacillus alvei* and the conception which Howard had of it, is in the fact that Howard thought that it grows better under anærobic conditions, while Cheshire and Cheyne obtained very satisfactory growth on the surface of media exposed to the air.

Propositions are stated by Howard in his paper, and his own interpretations of them are given. Some of his views can be accepted as good, others can not.

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[1] Holtermann, R. F., Monteith, S. N., Husband, E. M., 1893. Report of Apicultural Committee. Fifteenth Annual Report of the Ontario Agricultural and Experimental Union. Pp. 230-231. Contained in Nineteenth Annual Report of the Ontario Agricultural College and Experimental Farm.

[2] Howard, Wm. R., M.D., March 1, 1894. Foul brood; its natural history and rational treatment, with a review of the work of others. Chicago, Ill. Pp. 47.

His reference to the gross appearance of the disease material with which he was working strongly suggests that the disorder was American foul brood. In every case that he examined he reports the presence of *Bacillus alvei*.

Howard, September 10, 1896.

Having gotten Howard's conception of foul brood and *Bacillus alvei*, we shall pass to another paper [1] by the same writer.

The term "pickled brood," which is often used by bee keepers, had its origin apparently in an article by Howard in which he reported a condition in the brood of bees as a new disease. Since the term "pickled brood" is so frequently used in beekeeping literature it may be well to consider Howard's work somewhat in detail.

The trouble which he calls pickled brood he says had often been mentioned by writers in bee papers. Two years before his paper was written he himself had two colonies to die during the winter, and when in the spring the combs were examined they were found to be moldy, especially those containing pollen. These combs were given to other colonies with no bad results, until the brood which was being reared was about ready to be capped. By watching this brood he observed that it did not decay like "foul brood." When the larvæ are dead, he says, they have a swollen appearance, with neither end touching the cell as a rule. After a few days some of the larvæ settle down and change to a dark brownish mass which is watery, not ropy, and without odor.

From the combs and dead brood there was isolated a species of fungus to which he ascribed the cause of the trouble, and to which he gave the name *Aspergillus pollini*. Concerning his convictions as to the etiological relation existing between the fungus and the disease he writes: "Several experiments were made during the summer which fully satisfied me that my conclusions were correct." This condition suggested to Howard the possibility of its being the form of foul brood which responds to the shaking treatment and the drug method, and the form which disappears of itself when fresh pollen is consumed by the bees. He says that the fungus finds a good medium in food which contains pollen in the alimentary canal of the larvæ. The fungus, he says, breaks through the wall of the alimentary canal, permeates all the liquids of the body, and there produces acetic acid. The larva dies in about three days and is pickled in its own juices containing this acetic acid. Such a supposition suggested to Howard "pickled brood" as a name for the disease. On account of the acid reaction of the larvæ thus "pickled," he believed that no putrefactive germs entering from the air could grow

[1] Howard, Dr. Wm. R., September 10, 1896. A new bee-disease—pickled brood or white fungus. American Bee Journal, vol. 36, No. 37, pp. 577-578.

and cause putrefaction of the tissues, and for this reason no odor was present. He includes in his paper a differential diagnosis between foul brood and pickled brood.

In foul brood, he says that the brood is attacked at all ages, from two to three days after hatching until after it is capped, and that as much brood dies before the feeding of pollen as afterwards; that the brood is attacked by the putrefactive germs from the air, causing rapid decomposition, resulting in a ropy brownish mass that gives off a very foul odor; that the cappings of the sealed cells are usually ruptured by the gases generated within the cell, and that the larvæ are found in a shapeless mass lying on the lower side-wall of the cell and closely adhering to it. Furthermore, he says that when gelatin and potato are inoculated with a culture of *Bacillus alvei*, growth at once takes place, forming a viscid ropy liquid which gives off an offensive odor resembling foul brood, and when such cultures are exposed to putrefactive germs a growth of such bacteria takes place.

In pickled brood, on the other hand, he says that the brood is attacked only after the pollen is mixed with the liquid food, and it dies usually just before reaching the pupal stage, but that it may pass into this stage and be sealed before being overcome by death. No brood, he argues, dies before the age of feeding mixed food arrives. Being in this acid or pickled condition, the brood is not attacked by putrefactive germs, and, therefore, no decomposition takes place. There is a watery condition of the brood. The larvæ may be of a light brown color, but generally are white, and no odor is present. The capping is not ruptured in the brood that is sealed. The brood has a swollen appearance, does not stick to the cell wall, and often does not lose its shape. Furthermore, he argues that if brood is placed in a medium of sweetened water in which starch or wheat bran is mixed, and placed in a moist chamber within a dark room, growth of the fungus takes place and covers the surface of the medium. The medium becomes acid, and when such a culture is exposed to the air putrefactive germs do not attack it.

In this paper the following points are observed:

1. Howard used the term "pickled brood" for a disorder which was clearly different from "foul brood."

2. He gave a brief but fairly satisfactory description of the gross symptoms of the condition.

3. He claims that the disease is a specific infectious one.

4. He declares that the cause of the disease is a fungus which he isolated from larvæ dead of the disease and to which he gave the name "*Aspergillus pollini*."

5. The experimental data by which he was supposed to have proven that *Aspergillus pollini* is the cause of the pickled brood,

although not included in his paper, was, as he says, satisfactory to himself.

6. The description which Howard made of the fungus is not sufficient to identify it.

One at once observes that Howard brought forth no convincing evidence that the disorder with which he was working was infectious, nor that the fungus which he named *Aspergillus pollini* was a new one, nor did he prove that any etiological relation existed between the fungus and the disorder.

Inasmuch as the disorder which Howard described was declared by him to be due to a fungus, *Aspergillus pollini*, it is certain that the disease known to bee keepers as pickled brood is not the "pickled brood" of Howard. It is the opinion of the writers that the "pickled brood" described by Howard does not exist.

Howard, February 15, 1900.

Another paper[1] by Howard appeared in 1900, discussing still another disease which he supposed was not "foul brood." The disease, as will be learned later, was in all probability European foul brood.

He quotes a description of this disease by Mr. N. D. West, an able bee inspector of the State of New York. In this quotation Mr. West says that the disease appears in the spring about the time the apple is in blossom, breaking out all at once and spreading with amazing rapidity. The young larva has a yellow speck on the body, about the size of a pinhead. The older larvæ are lengthwise in the cell, white, and uncapped. After dying the brood is either removed by the bees or flattens out and becomes at first a cream-colored and later a coffee-colored mass. Later in the season some brood, which had died after capping, becomes coffee colored and of the consistency of heavy honey. When this is tested with a toothpick, the decaying mass stretches out to the extent of one-half inch to 1 inch. In some cases the odor is sour, while in others cases, especially if the capped stage has been reached by the dead brood, it has a somewhat rotten odor. The odor is not especially disagreeable at any time. The colony either dies out entirely from this disease, or the condition improves so that later in the summer no diseased brood can be found. With plenty of stores and a good flow, the disease seems to abate.

Mr. West's many years of experience as a bee keeper, his experience as an inspector of apiaries, and his ardent enthusiasm on questions relating to bee diseases, make his description of the appearance of this disease of much value.

Although Howard quotes from West the symptoms that are found in the so-called black brood, one finds him deviating far from them in

[1] Howard, Dr. Wm. R., February 15, 1900. New York bee disease, or black brood. Gleanings in Bee Culture, Vol. XXVIII, No. 4, pp. 121–127.

stating his own conception of the disease. In giving his own view of the symptoms of the disease, and in pointing out the differences which he supposed existed between "black brood," "foul brood," and "pickled brood," the writings of Howard indicate, as is evidenced by the following quotation, that he himself had a very inaccurate conception of the brood diseases of bees.

SYMPTOMS AND COURSE.

Brood is usually attacked late in the larval life, and dies during pupation, or later when nearly mature and ready to come forth through the chrysalis capping. Even after leaving the cell they are so feeble that they fall from the combs helpless. Most of the brood dies after it is sealed. In this it is much like pickled brood, except that as much or more brood dies in the late larval stage than in the pupa. In foul brood, while brood of all ages dies, yet more dies "at the ages of 6, 7, 8, and 9 days than at any other age" (author's Foul Brood p. 46), even before the rich chyle-like food mixed with pollen is given, which is such a necessary environment for pickled brood and *black brood.*

When the larvæ show the first signs of this disease, there appears a brownish spot on the body, about the size of a pinhead. The larvæ may yet receive nourishment for a day or two; but as the fermentation increases the brownish spot enlarges, the larva dies, stands out, swollen and sharp at the ends. In this they are like pickled brood, except that the brown spot is not present in pickled brood, but pickled brood sometimes becomes brown after death. Foul brood turns brown only after the action of the putrefactive germs have brought about decomposition. No decomposition from putrefactive germs takes place in pickled brood. In *black brood* the dark and rotten masses, in time, break down and settle to the lower side of the cells, as a watery, syrupy, granular liquid—not the sticky, ropy, balsam or glue-like semi-fluid substance of foul brood. It does not adhere to the cell walls like that of foul brood; has not the characteristic foul odor which attracts carrion-flies, but a sour, rotten-apple smell, and not even a house-fly will set her foot upon it. Cappings in foul brood are sunken in the center when broken, sometimes puffed out by internal gases. In *black brood,* the cap is disturbed from without, sometimes uncapped, and cell contents removed by the bees; not so in foul brood. The cap in pickled brood is usually undisturbed. The decayed brood masses do not adhere to the cell walls like either of the others.

The defect in Howard's description of the appearance of the brood which has probably caused the greatest confusion has reference to the color of the larvæ dead of the disease. In the following quotation he mentions the dark and black color of the brood, which according to him was so marked that it suggested the name "black brood."

On account of the character of the dead brood; its beginning with a dark spot on the larva, which increases in size, becomes darker, and finally black, for convenience and brevity the name *black brood* has been suggested, and this name is used in the text.

Since the so-called black brood is in all probability European foul brood, many bee keepers expect to find black larvæ in this disease. Occasionally some of the larvæ may be black, but black larvæ are seldom found. If the bee keeper will bear this fact in mind he will be very much aided in understanding the brood diseases of bees.

From samples of the so-called black brood, Howard reports that he isolated two new species of bacteria. The one he named *Bacillus*

milii and declared it to be the specific germ of "black brood"; the other he named *Bacillus thoracis* and thought probably that it modified the disease in some way. He said so little about either of these species that neither of them can be identified from his writings. In his examination of five specimens received from West, he reports *Bacillus milii* in all of them, *Bacillus thoracis* in two, and *Aspergillus pollini* in two.

In his experimental investigations Howard used two nuclei which were free from disease, and fed each of them a culture of "*Bacillus milii*" in one-half pint of sirup on November 7, and repeated similar feedings on November 10. The feeders were removed from each hive on November 12. When the bees were examined on November 26, no brood at all was found in one of the nuclei. In the other nucleus was found no eggs, but larvæ six or seven days old and considerable capped brood. Near the outer part of the brood-nest apparently some capped cells were found from which were taken nearly matured pupæ. Other pupæ, nearly white, with a dark spot on the abdomen, were removed, together with others which were dark or black all over. "*Bacillus milii*" was reported to have been found microscopically in nearly every pupa examined. Howard states that no uncapped larvæ seemed from gross appearance to be affected, but in the bodies of several of them *Bacillus milii* was found. The two nuclei were again examined on December 14. As before, there was nothing wrong apparently with one nucleus. In the other nucleus there were about 30 capped cells which contained dead pupæ that were nearly black. Microscopic examination caused him to report the presence of *Bacillus milii* in all of these pupæ.

It will be observed that the results which Howard obtained from the inoculation of the two nuclei with cultures of "*Bacillus milii*" neither proved nor disproved the causal relation of "*Bacillus milii*" to "black brood." As evidence to support his declaration that *Bacillus milii* is the specific cause of black brood, modified probably at times by *Bacillus thoracis*, he offers the data that *Bacillus milii* was found in all of the few samples which he examined, and that in a few instances *Bacillus thoracis* was also present.

In pointing out the differences between "foul brood," "pickled brood," and "black brood," the following three assertions are made by Howard; not one of them, however, has yet been demonstrated to be true.

Foul brood, pickled brood, and black brood. Foul brood, due to *Bacillus alvei*—a specific bacterium.

Pickled brood, due to *Aspergillus pollinis*—a specific fungus.

Black brood, due to *Bacillus milii*, modified, perhaps, by *Bacillus thoracis*, specific bacteria.

Summarizing this paper by Howard the following points might be mentioned:

1. Howard received during the year 1899 a few samples of a brood disease, principally through Mr. West, of New York State.
2. He reported that the disease was a new one and gave to it the name "New York bee disease" or "black brood."
3. From the samples of the disorder he claims to have isolated an organism to which he gave the name *Bacillus milii.*
4. He made no description of "*Bacillus milii*" by which it is possible to identify the organism positively.
5. He claims that "*Bacillus milii*" is the cause of the disorder which he studied, and in support of it he relates some experimental work.
6. He says that "*Bacillus milii*" may be accompanied by another species, "*Bacillus thoracis,*" which may assist in the destruction of the brood.

This concludes our consideration of three papers written by William R. Howard.

Reviewing the writings of William R. Howard, one learns that they have caused no small amount of confusion in the minds of bee-keepers respecting the brood diseases of bees. Howard claimed to have found *Bacillus alvei* in that form of foul brood characterized by a ropiness, and asserted that that organism is the cause of the disease. He gave the name "pickled brood" to an apparent disorder of bees which, he says, had often been mentioned in the writings of bee-keepers. He asserts that this "pickled brood" is due to a fungus to which he gave the name *Aspergillus pollini.* He called the disease about which Cheshire and Cheyne had already written (p. 25), "black brood." He declared the disorder to be due to a micro-organism to which he gave the name *Bacillus milii.* The incomplete description which Howard made of the species, however, does not make it possible to identify such an organism.

The authors of this paper have received some evidence, however, as to the identity of Howard's *Bacillus milii.* Howard sent bouillon and agar cultures of what he claimed was *Bacillus milii* to one of his correspondents stating that it was possible that the culture was not pure. Accompanying the cultures was a stained cover-glass preparation which he said was prepared from the vegetative form of the bacillus. The cultures, together with the stained preparation, were forwarded to us. In the cultures was found only *Bacillus alvei.* The stained preparation contained apparently only spores (not vegetative forms), and as far as it is possible to know from a microscopical examination these spores were the spores of *Bacillus alvei.* Such facts should dispel any particular anxiety one might possess concerning the existence of such an organism as *Bacillus milii.*

Harrison, December, 1900.

The next paper to be considered is one by Harrison.[1] In commenting upon the work of Cheshire and Cheyne, he asserts that these men established the causal relation of *Bacillus alvei* to foul brood by successfully applying Koch's rules in their work. He rehearsed in his paper the spraying experiment which Cheshire was supposed to have done and stated that since that time *Bacillus alvei* has generally been regarded as the cause of the disease.

In the beginning of his work, then, Harrison, like Mackenzie, accepted the work of Cheshire and Cheyne as conclusive that *Bacillus alvei* is the cause of foul brood. It is very unfortunate that these two men should have made this mistake, as their papers have had the effect of strengthening two erroneous ideas: First, that the two maladies which together were known as foul brood were one disease; and second, that *Bacillus alvei* is the cause of the disorder.

Harrison was aware of the fact that some bee keepers believed that there was more than one disease included under the name foul brood, since, in commenting on the work of Dickel and Klamann, he states that Dickel writes of one form of the disease which affects the unsealed brood, and of another form which affects the sealed, and even a third form, a mixed form which seems to be still more malignant. He states furthermore that Klamann suspected two kinds of disease.

Harrison entertained the following ideas concerning foul brood. The disease affects chiefly the larvæ, and when they are attacked they no longer lie curled up in the cell but are extended in it or move about unnaturally. The adult bees by a sort of inertness which seizes them may at this time show symptoms of the disease. The affected larvæ become flabby and die, and as a result of the decomposition which sets in, the decaying mass takes on a yellowish color. The yellow turns to a brown and when touched by a pin at this time or later, a portion of the mass may be pulled out in a long, ropy, tenacious string. This ropy mass dries down gradually to a brown scale which adheres to the wall of the cell. The bees as a rule are not active in removing larvæ dead of this disease from the cells, but, on the contrary, they are quite inactive, without desire to fly, but they may be seen fanning at the entrance of their hive. At this time a foul odor may be detected coming from the hive. The phase of the disease which some authors discuss as being a different form, Harrison states, is the same disease but that the larvæ die after being capped over instead of before. The capping of the cells containing such larvæ becomes indented or sunken and finally perforated. By

[1] Harrison, F. C., B. S. A., December, 1900. Foul brood of bees. Bulletin 112, Agricultural College and Experimental Farm. Pp. 32. Toronto. Portions of this bulletin are quoted in Bulletin No. 70, Bureau of Entomology, U. S. Department of Agriculture, pp. 23-26.

inserting a pin in either of these cells the same ropy mass may be drawn out. If an examination is made of the juices of the larvæ at different stages of the disease the bacilli may be seen. Spores form only after the death of the larvæ. The ropy decaying mass as well as the scales contain large numbers of these spores.

Weighing these facts it seems quite probable that Harrison was working with but one disease, American foul brood. In his examination of the ovaries of queens taken from foul-brood apiaries, Harrison reports the finding of bacilli in three queens. He reports that he found bacilli in a larger number of eggs laid in an affected colony, and writes:

> In view of these facts, I am of the opinion that the eggs of bees from diseased hives may in some instances be infected.

Harrison did not find *Bacillus alvei* in any case of chilled brood which he had examined and states that Mackenzie performed several experiments with chilled brood and never found this organism in any case where the brood had not been inoculated experimentally. Harrison writes as follows concerning the distribution of the disease:

> I have examined diseased larvæ from Canada, from Europe (France, Switzerland, Austria, Germany, Italy and England), Cuba, and 13 States of the Union, ranging from New York to California and from Michigan to Florida, and have succeeded in isolating *B. alvei* from all of them. It is true that some of the cultures show certain differences, but they have not been sufficiently pronounced to constitute even a well marked variety of the species.

Harrison may have isolated *Bacillus alvei* in limited numbers from material received from all these sources, but from his description of *Bacillus alvei* one can not be sure that he always identified his culture correctly.

It may be well at this point to consider Harrison's description of the organism which he identified as *Bacillus alvei*. In an abridged form it is as follows:

Occurrence.—Found in larvæ of bees suffering from a disease known as foul brood.

Gelatin plates.—The appearance of the growth depends upon the age of the colonies and character of the medium. In 24–36 hours at 22° C. the colonies are observed to be small, oval, or lozenge-shaped, bearing peculiar shoots extending frequently from one end and giving it a pear-shaped appearance. At the end of 48 hours the colonies are larger, with fine projections shooting out in all directions and forming circles. Later this appearance is destroyed by the liquefaction of the gelatin.

Agar plates.—In 12 hours at 37° C. the colonies are small and burr-like. Further, concerning the growth on agar, he writes:

> On agar plates streaked with a light inoculation, most beautiful forms occur. The growth of the bacilli spreads over the surface and branches repeatedly, giving the

appearance of seaweed. This appearance is distinctively characteristic; and as the growth is very rapid, this method commends itself for making a quick diagnosis of the presence of the bacillus in larvæ supposed to be diseased.

Morphology.—A slender bacillus with slightly pointed but rounded ends. They usually occur singly, but may form chains of various lengths. This species possesses a single flagellum at a pole. No capsule has been demonstrated.

Spores.—The spores are about 2 μ in length and 1 μ in breadth. Under favorable conditions they begin to germinate in about three hours.

Motility.—They are actively motile, especially in fresh cultures.

Oxygen requirements.—Harrison agrees with Cheyne (p. 31).

Bouillon.—In 14 hours at 37° C. there is a slight cloudiness, in 24 hours the culture is turbid, and in 48 hours the turbidity is further increased and a pellicle begins to form. After 96 hours the broth is rather clear, with a white, rather massive, and somewhat tenacious pellicle. Reaction of the medium after 10 days has changed from +0.08 to the neutral point.

Glucose.—Growth heavier in this medium than in plain bouillon. Reaction in 10 days is acid, having changed from +2 to +4.6. Involution forms may occur. They are slightly curved and average 5 μ in length.

Lactose.—The growth resembles that in plain bouillon, but is slightly heavier. Acid is produced but less in quantity than in glucose.

Saccharose.—Turbidity is greater than in any other bouillon and more acid is produced.

Potato.—Harrison writes:

> On potatoes the growth differs considerably, according to the reaction and age of the potato. Sometimes a brownish wrinkled growth forms, which gives off a peculiar odor; at other times a dryish yellow layer appears.

Milk.—At 37° C. coagulation of the casein occurs in three days. The medium becomes yellowish in color and gives off a peculiar odor. The coagulum finally digests, leaving a wheylike fluid.

Blood serum.—Growth takes place slowly. Many long filaments are common, which may be wavy and which may vary in thickness. The serum is liquefied.

Gelatin tubes.—On the surface in three days there occurs a ramifying growth. In five days the entire surface is liquefied. A whitish growth takes place along the line of puncture, from which numerous shoots branch out in the gelatin in all directions. This gives a haziness to the medium, which now begins to liquefy.

In the description which Harrison made he quotes rather freely from Cheyne. Some of his statements suggest that at least part of the time he was working with other species than *Bacillus alvei.*

One polar flagellum (p. 56) does not apply to *Bacillus alvei.* A heavy, tenacious pellicle does not suggest *Bacillus alvei.* The liquefaction in five days of the surface gelatin in tubes containing this medium does not suggest *Bacillus alvei.* The surface growth on agar, which Harrison describes as resembling "seaweed," is not encountered in the study of *Bacillus alvei.* These last three cultural characters are observed, however, in members of that group of bacteria of which *Bacillus A* (p. 76) is a member.

Harrison used cultures which he identified as *Bacillus alvei* in performing a number of laboratory experiments bearing directly upon the treatment of foul brood. His object was to determine the antiseptic value of various drugs for this species. The results obtained by this method of testing the antiseptic value were reported for weak solutions of salicylic acid, camphor, thymol, carbolic acid and tar, creolin, eucalyptus, beta naphthol, naphthalene, and formic acid. In doing this the drug to be tested was added to agar. The agar was inoculated with a pure culture of the organism, and observations were made as to whether a growth took place on this medicated medium.

Harrison gives the results of experiments in which he used two colonies of bees to test the value of naphthol and formic acid in the treatment of foul brood. The results which he obtained, however, can have only a relative value in treatment, since the organism with which he worked has most likely no etiological relation to any disease, or at least an unimportant one. Harrison had reached the conclusion that considerable attenuation in cultures of *B. alvei* may take place by its prolonged growth on artificial media. Since old cultures on this ground might be objectionable, he used, in his experimental inoculation, cultures which were only three generations from the diseased larvæ.

The further technique, in the carrying out of his experiment, involved the feeding of the spores from cultures to each of two healthy swarms placed side by side. The spores were scraped from the surface of an agar slant, put into 10 cc. of water, and well shaken. This suspension was then poured into medicated sirup. One colony was fed sirup containing the spores of *B. alvei* and one-third of a grain of beta naphthol to 1 liter of the sirup. The other colony was fed sirup containing the spores of *B. alvei* to which about 1.8 cc. of formic acid to a liter of sirup had been added. In both cases the medicated and inoculated sirup was taken up readily by the bees. The feedings were continued for three weeks, feeding four times per week. Each colony received in this way the growth from 12 agar slants. During the feeding period the combs containing the brood were examined, but no typical symptoms of the disease appeared. Cultures of *B. alvei* were obtained, however, from different parts of

the hive and from the digestive tract of the workers. After the third week, to each colony the feeding of ordinary unmedicated sirup containing the spores of *B. alvei* was practiced. In each experimental colony typical symptoms of the disease are reported to have been observed in 10 days, and a well-established disease after 16 days.

This experiment is offered as proof by Harrison that the feeding of an antiseptic in the treatment of foul brood is beneficial, as it hinders the germination of the spores of *B. alvei*. This confirms, he states, the opinion of Lortet that the digestive canal of the nurse bee is alone affected. Harrison reports the finding of *B. alvei* in the digestive canal of adult bees taken from diseased colonies.

After giving the results of his experiments, Harrison writes the following conclusion:

> From the results of the above experiments I conclude that in certain cases the use of chemicals is beneficial, but I would not say that other measures, such as starvation and stamping out, should be abandoned as unnecessary or useless. Some of the drugs used are of very little, if any, value; but others, such as formic acid and napthol B, are undoubtedly very useful. In some cases, especially those in which the disease is very virulent, it may be advisable to resort to more drastic measures.

In another experiment he reports that symptoms of the disease were produced after 14 days when *B. alvei* was fed. In these inoculation experiments, cultures were used which had been recently isolated in order that the virulence might not be diminished. He reports one experiment, however, in which cultures were used which had been transferred 30 times, with the result that several weeks elapsed before the disease appeared and then only in a light form.

One observes here that Harrison reports at least four cases in which foul brood developed after feeding the spores of *B. alvei* in sirup. These are of special interest inasmuch as many failures have been made since that time to obtain the symptoms of foul brood by similar inoculations. It would be well if Harrison could repeat these feeding experiments for confirmation.

The following summary contains some of the features of interest in Harrison's paper:

1. It has now been 11 years since the bulletin by Harrison, which is here briefly reviewed, was published, and very naturally, as its author no doubt will agree, it is in need of revision.

2. He has given in the historical résumé a brief account of the results and beliefs of a number of workers and writers on foul brood.

3. He believed that the two forms of foul brood described by some authors were only two phases of the same disease, one form being that phase of the disease in which the larvæ die just before capping, and the other one that phase of the same disease in which the brood dies after capping.

4. He gives a geographical distribution of foul brood, having determined it by the isolation of *B. alvei* from diseased larvæ.

5. He reports the finding of *Bacillus alvei* in the ovaries of queens, and concludes that the eggs in diseased hives might sometimes be affected.

6. He has given quite a lengthy description of *B. alvei.*

7. He was not able to isolate *B. alvei* from chilled brood as the condition is found in the apiary.

8. He performed various experiments to prove the value of drugs in the treatment of foul brood, with the conclusion that drugs in certain cases—for example, formic acid and beta-naphthol—are undoubtedly very useful, while some others are of very little or no value.

9. He also reports the successful production of the disease with typical symptoms by feeding the spores from pure cultures of *B. alvei.*

10. He mentions no difficulty in obtaining *B. alvei* from "foul-brood" material.

The authors of this bulletin disagree with nearly all of the points made by Harrison. His failure to recognize the fact that the mass of spores, which are always seen in brood dead of American foul brood, do not grow when plate cultures are made, was fatal to his work. Occasionally colonies of *Bacillus alvei* do appear on plates, made from this disease, but when they do they appear only in relatively small numbers.

LAMBOTTE, SEPTEMBER 25, 1902.

A paper by Lambotte [1] caused, at the time it was written, considerable discussion among bee keepers. It caused some comment also on the part of others on account of the views which he expressed concerning the identity of *Bacillus mesentericus vulgaris* and *Bacillus alvei,* and the relation of *Bacillus mesentericus vulgaris* to foul brood.

In view of the fact that foul brood seemed to appear unexpectedly away from all known sources of infection, a beekeeping society petitioned the minister of agriculture of Belgium to have a new scientific study of foul brood made. Lambotte's work is the result of this petition. From requests made through journals, an ample supply of foul-brood samples was received. The diseased larvæ in capped cells were recognized by the darkened, sunken, and usually perforated cappings. The brood dead of the disease he describes as being yellow or brownish-yellow, viscid, ropy, and emitting a nauseating odor.

From a stained preparation made from ropy larvæ, Lambotte observed a very large number of spores which he supposed were the

[1] Lambotte, Dr. Ul., September 25, 1902. Recherches sur le microbe de la "loque," maladie des abeilles. Travail du laboratoire de l'institut de pathologie et de bacteriologie de l'université de Liége. Annales de l'Institut Pasteur, Tome XVI, No. 9, pp. 694-704.

spores of *Bacillus alvei*. When he inoculated gelatin, agar, or bouillon with some of this ropy larval mass, no growth took place and when he used larvæ which were more recently attacked, the same failure to obtain a growth was observed. He took these facts to mean that there is something present in the foul-brood larvæ which prevents, by its antiseptic property, the growth of the bacteria. He proceeded, therefore, to inoculate a considerable quantity of sterile bouillon, in order that the supposed antiseptic present might be diluted, thus permitting the germination of the spores. By following this technique a growth was obtained and he interpreted it to be the growth of the spores which he had observed microscopically in such large numbers in the diseased brood.

The bacillus which he thus obtained he isolated in a similar manner from a large number of samples of "foul brood," and by a study of its morphology and cultural characters Lambotte identified it as being the one described by Cheyne as *Bacillus alvei*. Furthermore, he studied the morphology and cultural characters of a number of cultures of *Bacillus mesentericus*, and by a comparison of these with those of *Bacillus alvei* he reached the conclusion that the two are very similar.

To show more conclusively that *Bacillus alvei* and *Bacillus mesentericus* are very similar, Lambotte made use of the phenomenon of agglutination. Guinea pigs were used in his experiments. In immunizing the animals he used a suspension of an agar culture of *Bacillus alvei* and *Bacillus mesentericus*, respectively, in physiological salt solution. In each case the animal received four inoculations at weekly intervals. He reports that the serum of an untreated guinea pig did not exhibit, upon examination, the phenomenon of agglutination. The serum of a guinea pig, on the other hand, which had been treated by inoculations with *Bacillus alvei*, agglutinated a culture of this species at a dilution of 1 to 350, and the serum from the same animal agglutinated cultures of *Bacillus mesentericus* at a dilution of 1 to 250. Furthermore, the serum of a guinea pig that received the inoculation of *Bacillus mesentericus* agglutinated *Bacillus mesentericus* as well as *Bacillus alvei* at a dilution of 1 to 250. Neither of these two sera agglutinated any other bacilli at these dilutions. This caused Lambotte to conclude that *Bacillus alvei* and *Bacillus mesentericus vulgaris* are the same species.

Having convinced himself that this very intimate relation exists between *Bacillus alvei* and *Bacillus mesentericus vulgaris*, he attempted to prove by the inoculation of healthy colonies that foul brood could be produced with cultures of *Bacillus mesentericus*. He killed some of the larvæ by pricking them and placing a suspension of *Bacillus mesentericus* in the cell with the dead larvæ. He hoped

that foul brood might be started in the colony, but repeated attempts resulted each time in failure.

He then made a medium from bee larvæ by the use of which he thought he had obtained by successive inoculation a special variety of *Bacillus mesentericus*. He supposed that if *Bacillus mesentericus* were grown upon the bee-larvæ medium its virulence for bees would be increased. After using the culture of *Bacillus mesentericus*, which had been grown upon the bee-larvæ medium, and after inoculating larvæ as before, he reports that foul brood was produced with the typical symptoms of the disease. The only exception noted was that fewer cells were affected.

He attributed his good results to two facts, first, that *Bacillus mesentericus* was cultivated on a bee-larvæ medium, and second, that the experimental inoculation was made at a time of the year when the activity of the hive was considerably diminished. To the latter factor he attributes most of his success. He states that a colony inoculated with *Bacillus alvei* or with *Bacillus mesentericus* grown on a bee-larvæ medium will not allow itself to become infected when it is active at the beginning of the season.

In his conclusions Lambotte writes:

(1) *Bacillus alvei*, described by Watson Cheyne and Cheshire as the specific cause of foul brood, is simply the widely distributed organism *Bacillus mesentericus vulgaris*.

(2) *Bacillus mesentericus* can be found in healthy hives, in the cells of the comb, and in the intestines of bees.

(3) *Bacillus mesentericus*, by growth in the tissues of larvæ, produces changes characteristic of foul brood.

Lambotte insists that the hygiene of the colony is above all the most important in the control of foul brood. He believes that unless the resistance of the larvæ to infection is maintained by good hygiene, *Bacillus mesentericus*, which is so widely distributed in nature, may invade the colony and produce foul brood in any apiary.

A brief summary of Lambotte's works may be made as follows:

1. He did not take into consideration the two forms of foul brood described by some authors, working for the most part, at least, with American foul brood only.

2. He observed that in American foul brood it was not easy to obtain a growth of the spores which are found in such large numbers on microscopic examination.

3. He suspected that an antiseptic was present in the dead remains of the larvæ in this disease which prevented the growth of the spores. The effect of such an antiseptic he hoped to overcome by diluting it with a large amount of the medium used for its cultivation.

4. He obtained a bacillus by this special technique and identified it as the *Bacillus alvei* of Cheyne and Cheshire.

5. He compared *Bacillus alvei* and *Bacillus mesentericus vulgaris* and arrived at the conclusion that they are one species and offered as proof of it that the morphology and cultural characters of the two are similar, and that the serum of an animal immunized with *Bacillus alvei* will agglutinate *Bacillus mesentericus vulgaris* and vice versa. Furthermore, he claimed that foul brood can be produced with cultures of *Bacillus mesentericus vulgaris.*

6. He believed that when the resistance of the larvæ is for any reason lowered *Bacillus mesentericus,* if introduced, can become virulent and produce "foul brood." In this way he explained the presence of "foul brood" in an apiary without the introduction of infective virus from without.

This work of Lambotte has been criticised by different writers since its appearance. The spores which he observed to be difficult of germination were most likely not caused to germinate by the technique which he used. It would seem also that he was in error in concluding that *Bacillus mesentericus* and *Bacillus alvei* are one species. This conclusion led him to the unlikely supposition that "foul brood" might appear in any apiary without the introduction of an infective virus other than the widely distributed and commonly met with organism *Bacillus mesentericus vulgaris.*

Harrison, February 28, 1903.

In a review, Harrison[1] disagrees with some of the views expressed in Lambotte's paper (p. 53). He did not believe with Lambotte that *B. alvei* and *B. mesentericus vulgaris* are one species. It was his opinion that Lambotte's work on these two species was insufficient to establish their identity. Harrison compared the descriptions of the two species made by different authors and offered the results as evidence that the two were different. In offering the evidence he states that he did not have time himself to make, for comparison, a study of the cultures themselves. Harrison was led to believe that Lambotte began his experiments with *Bacillus mesentericus vulgaris* and not with *Bacillus alvei.*

Two minor points of considerable interest are also recorded: First, Harrison at this time states that he too had had at times some difficulty in obtaining a growth from the spores in "foul brood"; and second, he now credits Cowan for having said that *Bacillus alvei* possessed but one flagellum.

The following sentence from Harrison's paper is offered as an argument to disprove the identity of *Bacillus alvei* and *Bacillus mesentericus vulgaris:*

[1] Harrison, F. C., February 28, 1903. *Bacillus mesentericus* et *B. alvei.* Revue Internationale d'Apiculture, Tome XXV, No. 2, pp. 29–32.

If Dr. Lambotte's theory that *Bacillus mesentericus vulgatus* and *Bacillus alvei* are identical is true, we should naturally expect to find cases of foul brood arising spontaneously in countries which never import bees or material from infected localities.

This assertion could be admitted as evidence if *Bacillus alvei* were known to have as important an etiological relation to foul brood as Harrison supposed that it had (p. 48). One of the facts which prompted Lambotte's investigation was that the disease seemed to break out independently of any source of infection. If any casual relation does exist between *Bacillus alvei* and any form of foul brood, and if his theory concerning the identity of *Bacillus alvei* and *Bacillus mesentericus vulgaris* can be established, Lambotte offered a very clever explanation for the existence of the apparently sporadic cases of the disease.

It is not likely that Lambotte produced foul brood with either *Bacillus alvei* or *Bacillus mesentericus vulgaris*. The two species are most likely different species, but the evidence advanced by Harrison to prove that the two are different is inferior to that which Lambotte produced to establish their identity. Lambotte made his greatest error apparently in faulty observations.

In the opinion of the writers, both Harrison and Lambotte were probably describing their experiences with American foul brood, and the spores which they saw in such large numbers were those of *Bacillus larvæ*. The growth which they obtained was not a growth from these spores, but a growth of *Bacillus alvei* or a member of the group to which *Bacillus mesentericus vulgaris* (*Bacillus A*, p. 76) belongs. Either of these species may be present but occur most always in small numbers.

Another point of considerable interest might be mentioned here. In a paper by Harrison[1] presented to the Bee Keepers' Association of the Province of Ontario in November, 1904, the following is found:

Two years ago I remember there was some talk of black brood, and I think a committee was appointed to send samples to me. Whether they did not meet with any cases of black brood or no I don't know, but I know I have received no samples, * * * .

This statement tends to confirm the suspicion already expressed that Harrison was working with American foul brood.

In an address[2] delivered in November, 1905, before the Bee Keepers' Association of the Province of Ontario, Harrison announced to the association that he was leaving his position as director of the association and representative of the agricultural college. Since that time we have not learned of any work on bee diseases published by

[1] Harrison, F. C., 1905. Diseases of bee larvæ. Annual Report of the Bee Keepers' Association of the Province of Ontario, 1904. Toronto, pp. 27–36.

[2] Harrison, F. C., 1906. Diffusing apicultural knowledge. Annual Report of the Bee Keepers' Association of the Province of Ontario, 1905, pp. 8–10. Toronto.

him. It is to be regretted if the duties in his new position limit his activity in this line of research.

Moore and White, January 15, 1903.

In the spring of 1902 Moore, assisted by one of the writers of this bulletin, began the investigation of the bee diseases that were present in the State of New York. Neither was familiar with the manifestations of the different diseases attacking bees, further than the information which could be obtained from the publications of Cheshire and Cheyne, Howard, Harrison, and data obtained directly from the four inspectors of apiaries of that State—Messrs. West, Stevens, Stewart, and Wright. The samples of brood examined were received from these inspectors with the diagonsis of each sample already made. The first report [1] which was made to the commissioner of agriculture of that State on the investigation gives a brief account of the examination of 10 samples labeled "black brood," 7 samples labeled "foul brood," and 5 samples labeled "pickled brood."

William R. Howard, it will be remembered (p. 44), received samples of diseased brood from N. D. West, of New York. He examined them and reported the disease as new, naming it "New York State bee disease" or "black brood." He ascribed the cause of the disease to a bacillus, to which he gave the name *Bacillus milii*. The 10 samples labeled "black brood," and examined by the authors of the paper under consideration, were all from New York State and 7 of them were diagnosed by Mr. West.

The bacterial findings recorded by Moore and White show that *Bacillus alvei* was present in all the samples, while there is no record of *Bacillus milii* in any of them. Other bacteria, which appeared in most instances to be micrococci, were occasionally associated with *Bacillus alvei*. The absence of any bacillus corresponding to the description of *Bacillus milii* in these samples of so-called "black brood" was strong evidence that such a species was not the cause of the disorder.

The question naturally arose as to whether this trouble was a new disease, as Howard had led the people to believe. In forming an opinion as to whether a new disease existed, the work of Howard and others was considered. Cheshire and Cheyne (p. 25) had described the symptoms of "foul brood" and had apparently found *Bacillus alvei* present in sufficient numbers to suspect this species as the cause of the disease. Harrison (p. 48) had found a species in "foul brood" which he had identified as *Bacillus alvei*. Lambotte (p. 53) had done

[1] Moore, Veranus A., M. D., and G. Franklin White, B. S., January 15, 1903. A preliminary investigation into the cause of the infectious bee disease prevailing in the State of New York. State of New York, Department of Agriculture, Tenth Annual Report of the Commissioner of Agriculture, for the year 1902, pp. 255–260, two plates.

likewise. Ten samples of diseased brood were therefore examined which corresponded in gross appearance to the symptoms of the disease which Cheshire and Cheyne reported, and the bacteriological examination of these 10 samples revealed the presence of *Bacillus alvei* in numbers sufficient to lead one to suspect a causal relation of the organism to the disease. The conclusion that would be drawn from these facts regarding the disease, in the absence of Howard's work, very naturally would be that it is not a new disease, but simply "foul brood."

It was necessary now to weigh the evidence which Howard produced in support of the view that the disease was new. Howard received 5 samples from Mr. West and reported the presence of "*Bacillus milii*" in all of them, "*Bacillus thoracis*" in 2, and "*Aspergillus pollini*" in 1, but nothing was found in the samples of so-called "black brood" received and examined by Moore and White which could be identified as either species. The experimental data which Howard offered in support of his view was also very unsatisfactory.

In view of all these facts the authors of the report under consideration drew the conclusion: "That the prevailing disease [so-called black brood] in this State [New York] is very similar to, if not identical with, the 'foul brood' of other States, Canada, and Europe." This conclusion means that the disease, which the people of New York State were taught by Howard to be a new disease and which he chose to call "The New York bee disease" or "black brood," is not a new one, but is the one which Cheshire and Cheyne agreed was "foul brood."

Various inoculation experiments were now tried for the purpose of demonstrating whether or not *Bacillus alvei* is the cause of a brood disease. Several methods of inoculation which had been used by others in attempting to produce the disease in healthy brood experimentally were employed, but always with negative results. The most logical way to make the inoculation and the one which might be expected to give the most accurate results, very naturally, is by feeding the bees. This was tried with the hope that it would give the most accurate results.

The inoculation of one colony only is reported. A colony free from disease was fed, on August 4, sugar sirup, to which was added the spores of *Bacillus alvei* taken from agar plates and the vegetative form of the same species taken from fresh bouillon cultures. Similar feedings were given to the bees three times per week until September 28, but the symptoms of "black brood" did not appear. The results of this experiment, therefore, were negative, as were all the others as far as the producing of the disease was concerned. To make sure that some of the culture fed had reached the larvæ, cul-

tures were made from the larvæ before feeding and after feeding. *Bacillus alvei* did not appear on the plates made before the colony had been fed the culture, while many appeared on those made after the cultures were fed. The culture therefore reached the larvæ and no disease resulted. While the negative results of these few experiments were not sufficient to disprove an etiological relation between *Bacillus alvei* and foul brood, it did cause those doing the work to question the experimental results which others had reported.

The samples of American foul brood which were received and examined were labeled "foul brood." Six of the seven samples of this disease received gave no growth when cultures were made. Concerning the bacteriological findings in this disease the following is written:

> Stained cover glass preparations made from the dried dead larvæ contained large numbers of spores, but they failed to grow in any of our media.

Since the spores which were found in such large numbers in the larvæ dead of this disease would not grow, no inoculation of healthy brood was attempted. The samples of this disease did not show, on examination, the presence of *Bacillus alvei*. This at once suggested the fact that the disease is not the one studied by Cheyne as foul brood.

A study of five samples of the so-called pickled brood gave practically negative results both microscopically and culturally. The point to be noted here is that no fungus was found in this disorder corresponding to the one described by Howard (p. 42) as *Aspergillus pollini*. This fact very naturally suggested the probability that Howard had made another error in the determination of the cause of a brood disease.

The report included the study of brood from only three healthy apiaries. In samples taken from two of them, *Bacillus alvei* was not found, while a sample from the third apiary which was thought to be healthy but in a diseased district, showed the presence of *Bacillus alvei* in considerable numbers.

The following facts were learned in the investigations just reviewed:

1. At least two infectious diseases affecting the brood of bees exist. This fact had been known, however, for some time by the inspectors of apiaries of New York State, and by Dzierzon (p. 18) and many others years before.

2. Howard had erroneously reported European foul brood to be a new disease, which he named the "New York bee disease" or "black brood."

3. To produce foul brood in a healthy colony by feeding cultures of *Bacillus alvei* was by no means easy.

4. The results reported by others regarding the causal relation between *Bacillus alvei* and "foul brood" were questionable.

5. The spores found in the brood dead of American foul brood would not grow on the media commonly used in the laboratory, suggesting the possibility of a new and interesting species.

6. The disease which was being diagnosed as "foul brood" is not the foul brood studied by Cheyne, but is another disease now known as American foul brood.

7. *Aspergillus pollini* was not present in the samples labeled "pickled brood," meaning that the condition was not Howard's "pickled brood," or that he had made another error in his study of the condition.

8. No colonies of *Bacillus alvei* appeared on the plates made from the brood of two healthy apiaries, but colonies of that species did appear in considerable numbers on plates made from brood taken from a third apiary which was considered healthy, but located in an infected district.

9. Lastly, much work would have to be done on bee diseases before the confusion could be cleared up and the causes demonstrated.

WHITE, JANUARY 15, 1904.

In 1904 another paper[1] appeared giving the results of some investigations on bee diseases made during the summer of 1903. The work was a continuation of that done the preceding year.

It was desirable to know whether *Bacillus alvei* was constantly present in the samples of the so-called black brood. If this species could be found in large numbers in every sample of this disease and not in other conditions, it would be a valuable means of diagnosing the disease as well as suggesting a possible etiological relation between the organism and the disease.

Toward establishing the constant presence of *Bacillus alvei* in the foul brood of Cheshire and Cheyne (the so-called black brood) 26 samples were examined and *Bacillus alvei* was found in all of them. Twenty-four of the 26 samples were sent by Mr. West, but no species was found in any of them which was suspected as being *Bacillus milii*. These bacteriological findings, therefore, corroborated the conclusion of the preceding year that the so-called black brood is simply the foul brood of Cheshire and Cheyne.

Inasmuch as the efforts to produce foul brood with cultures of *Bacillus alvei* failed to give positive results in 1902 (p. 59), further attempts were made to determine the pathogenesis of this species for

[1] White, G. F., January 15, 1904. The further investigation of the diseases affecting the apiaries in the State of New York. State of New York, Department of Agriculture, Eleventh Annual Report of the Commissioner of Agriculture, for the year 1903, pp. 103-114.

bees. In the feeding experiments of this year some of the brood died. This slight difference in the results, from those obtained the preceding year, was probably due to the difference in the details of the experiment. Although some of the brood was found dead, the condition did not present sufficient symptoms, in the opinion of the author, to justify him in pronouncing it foul brood. Many punctured cappings were observed and dead larvæ of a dull color were present. These dead larvæ were found by cultures to contain *Bacillus alvei*. The death of these larvæ might have resulted from chilling or other causes, and the cappings may be punctured by the bees in different conditions that result in the death of the brood after capping.

The presence of *Bacillus alvei* could easily be explained from the fact that cultures can be isolated from apparently healthy larvæ taken from a colony to which the spores of the species has been fed. There was also wanting in these dead larvæ the yellowish color usually observed in those affected with European foul brood. The slightly viscid character which is sometimes present in brood dead of European foul brood was also absent. The rapidity with which the condition disappeared when the days became warmer was another indication that the disease was not European foul brood. The results of the experimental inoculation of healthy brood with cultures of *Bacillus alvei* were negative, and were therefore similar in this respect to those of the preceding year.

Since a number of articles had appeared about that time advocating the use of formaldehyde gas in foul-brood treatment, some preliminary experiments were conducted to test the efficiency of this disinfectant when applied to brood combs. The experiments demonstrated that it is not easy to destroy the spores which are within the dead larvæ and that the gas as it was being applied in the treatment of the brood diseases could not be relied upon.

The samples of American foul brood which were received for examination in 1902 were labeled "foul brood" when received. From the time Cheshire and Cheyne published their joint paper in 1885 to 1902 *Bacillus alvei* was suspected as being the cause of "foul brood." *Bacillus alvei*, however, was not encountered in the samples labeled "foul brood" received and studied in 1902 (p. 60). Spores were found present in the decaying remains of the dead brood, but they refused to germinate on artificial media.

The first time that these spores were caused to germinate under laboratory conditions was in 1903. For this purpose a special agar medium was used, made from the larvæ of bees. A somewhat similar medium had been used by Lambotte (p. 55), but with it he did not obtain a germination of these spores. This special agar was used in a test tube, and Liborius's method for making inoculations was

employed. Until the organism could be further described, and until there was more evidence that there was a causal relation existing between the species and the disease with which it was found associated, it seemed best to refer to the bacterium as *Bacterium "X"* and to the disease as "X brood." Seven samples of this disease were studied in 1903, and *Bacterium "X"* was found by cultures in all of them.

The disease called "pickled brood" received some further study at this time. The most striking feature in the results was the record of no growth from the cultures. The following is taken from the report:

The results of the examinations showed that "*Aspergillus pollinis*" was not found. Further investigations must be made before any conclusion can be drawn as to the real cause of this trouble.

Concerning paralysis in adult bees, the following was written:

The disease known to the apiarists as palsy or paralysis attacks the adult bee. The name is suggestive of the symptoms manifested by the diseased bee. A number of bees affected were received from Messrs. Wright and Stewart taken from apiaries in New York State. Bacteriological examinations have been made of a number of the bees so affected but no conclusions can be drawn from the results thus far obtained as to the cause of this disorder.

The following is a brief summary of the results obtained during the year 1903:

1. *Bacillus alvei* was found in all samples of European foul brood examined.

2. A causal relation between *Bacillus alvei* and European foul brood seemed questionable.

3. *Bacillus alvei* was not encountered in any sample of American foul brood.

4. The samples of American foul brood did contain, however, a species which was referred to as *Bacterium "X,"* in such numbers and with such constancy as to suggest an etiological relation to the disease.

5. A growth of this species was obtained on artificial media.

6. Neither "black brood" nor "*Bacillus milii*" was found. The work of the year seemed to confirm the idea that the so-called "black brood" was simply the foul brood of Cheshire and Cheyne.

7. The cultural results obtained from the so-called pickled brood were practically negative.

8. The "*Aspergillus pollini*" named by Howard was not found in any disorder of the brood of bees.

9. A disease called palsy or paralysis by the bee keepers seemed to be a malady, but no cause was found.

10. Formaldehyde gas as ordinarily used in the apiary would not insure complete disinfection.

Bahr, 1904.

A paper on the diseases of bees by Dr. L. Bahr,[1] of Denmark, bears the date 1904. The author gives a brief review of the work on bee diseases, together with some interesting observations by himself. In that portion of his paper describing his own observations the following is recorded:

> A number of samples of brood have been sent to me from various parts of the country (Denmark) having the following symptoms: Some of the diseased larvæ were quite small, while some of them are older—from 4 to 6 days. They never become ropy as those of foul brood, but retain their form until they approach the consistency of gruel. The color is whitish yellow but sometimes somewhat darker. In the gruel-like mass of the diseased larvæ I found a very small oval bacterium.

Bahr mentions that the disease seems to be quite contagious. From his description of the disease and from his bacteriological findings there is a strong suggestion that the disease to which he refers is European foul brood. Sufficient facts, however, are not given to make this point at all positive. The author states that his studies were not completed.

Burri, October and November, 1904.

We shall now consider a very excellent piece of work on foul brood by Dr. Burri.[2] In his introductory remarks this author very aptly refers to the need and value of a scientific study of foul brood.

Burri began his work on foul brood apparently in the spring of 1903. He observed that the foul odor which is emphasized so much in the literature on "foul brood" is not constant for all samples. Studying the different samples he concluded that the ropiness of the decaying larvæ and the tonguelike scales on the lower side wall of the cell were characteristic of typical "foul brood."

He also calls attention to the very large number of spores in the decayed foul-brood larvæ, and the absence of any vegetative forms. Cultures were made from these dead larval remains, but there was no germination of the numerous spores. The occasional colony which did appear he attributed to an accidental contamination with a different species. Failing in his attempt to obtain a growth of these numerous spores, Burri came to the conclusion that they were a new species that would not grow on the media ordinarily used in the laboratory. He added to his medium some cooked healthy larvæ somewhat similar to the medium used by Lambotte, but with this special medium he did not obtain the growth desired. Failing still to obtain a growth of the species, he proceeded with the study of its morphology as observed in the various stages of decay of the brood.

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[1] Bahr, L., 1904. Vore Bisygdomme. Foredrag holdt ved DBF's diskussionsmøde i Grejsdalen den 11 Septbr. 1904. Saertryk af Tidsskrift for Biavl Nr. 16 og 17.

[2] Burri, Dr. R., October and November, 1904. Bakteriologische Forschungen über die Faulbrut. Schweizerische Bienenzeitung, Nro. 10, pp. 335-342; Nro. 11, pp. 360-365.

Further attempts were then made to study the species culturally. He smeared some freshly infected larvæ supposed to contain only rods upon a certain medium and obtained spore-bearing rods and spores similar to those which had been observed in the diseased larvæ. He made a similar inoculation from the dead larvæ which had turned brown and which contained only spores, and as a result of this inoculation he obtained motile rods which later formed spores. Burri was somewhat inclined to believe that pure cultures had been obtained by his method of inoculation, although he states that the obtaining of pure cultures of this organism had to remain an unfulfilled wish.

From his studies Burri came to the conclusion that the organism is neither *Bacillus alvei* nor *Bacillus mesentericus*, but a new one. He repeated some of the experiments reported by Lambotte (p. 53), but was inclined to believe that the latter was in error. Besides studying from a number of samples this form of foul brood to which he referred as the nonstinking form (most probably American foul brood), Burri received and studied other samples of foul brood to which he referred as the foul-smelling form (most probably European foul brood). In the latter disease he found a large number of bacteria unlike those observed in samples of the other disease studied. The species which was present in large numbers in the latter samples grew without difficulty when sown on artificial media, and he identified it as *Bacillus alvei* Cheshire and Cheyne (p. 25).

We are not inclined to think of this latter disease (European foul brood) as the one which is the more foul smelling of the two, nor the former the ropy form (American foul brood) as the less stinking one of the two. It is true that only a few of the samples of American foul brood have a disagreeable odor when they reach the laboratory; nevertheless, the most disagreeable odor encountered in diseased brood when it is examined in the apiary is present in those colonies that are affected with American foul brood. It is American foul brood that the American bee keepers think of when they refer to the foul-smelling foul brood.

Burri encountered other bacteria than *Bacillus alvei* and the one which was difficult of cultivation. He mentions the presence of bacteria which he associated with a condition referred to as sour brood. He reports that he had always found foul brood present with this latter condition.

The following are the conclusions drawn by Burri in his paper:

1. There are in Switzerland, and also in other places, at least two distinct kinds of bacteria which can produce a typical contagious foul brood. In one case it is *Bacillus alvei* described by Cheshire and Cheyne; in the other a species of bacterium not formerly known, which is difficult to cultivate.

2. The two kinds of foul brood are easily distinguished from each other in the dried remains of the larvæ. That form of the disease in which *Bacillus alvei* is found exhibits an offensively smelling residue in which microscopically are found rods 2 μ in length, together with numerous spores. The larval remains in which are found the organism that is difficult to cultivate are almost odorless, and on microscopic examination spores 15 μ in length are recognized, but no rods.

3. Occasionally other bacteria which stand in a certain relationship to the so-called foul-brood germs obtain local significance as the cause of foul brood. Lambotte's view, on the other hand, that the potato bacillus (*B. mesentericus vulgatus*) is to be considered the cause of foul brood is yet without demonstration.

4. In choosing the methods for eradication of the disease, the fact that there are at least two kinds of foul-brood bacteria must be taken into consideration.

5. In every case a certain amount of knowledge of the bacteria in question is desired, not only from the scientific but from the practical point of view as well.

Some of the interesting facts noted in Burri's paper might be summarized as follows:

1. He recognizes two forms of foul brood.

2. He refers to the ropy type of foul brood (American foul brood) as the non-smelling form of the disease, and to European foul brood as the foul-smelling form.

3. He did not obtain a growth of the spores present in American foul brood either on the media ordinarily used in the laboratory or on a special medium to which cooked bee larvæ were added.

4. He studied the morphology of the organisms present in the foul-brood larvæ in a manner similar to that followed by Cheshire (p. 19).

5. He expressed doubt concerning the accuracy of the results reported by Lambotte.

6. In one disease (probably European foul brood) he obtained *Bacillus alvei* in very large numbers.

7. He found a condition to which he referred as sour brood, and with it he found associated a species to which he referred as sour-brood bacteria.

8. In his investigations he says foul brood always accompanied sour brood.

White, January 14, 1905.

The work on bee diseases was continued during the year 1904 in New York State and was followed by another report.[1] The work of the year was devoted to the diagnosis of the brood diseases in the laboratory; to a study of "foul brood" (European foul brood) and "X" brood (American foul brood); and to a study of palsy or paralysis in bees.

The similarity that exists between samples of the different brood diseases was observed to be so marked at times that a diagnosis of a condition often could not be positively made without a bacterio-

[1] White, G. Franklin, January 14, 1905. State of New York, Department of Agriculture, Twelfth Annual Report of the Commissioner of Agriculture, for the year 1904, pp. 106–107.

logical examination. This called for considerable work in diagnosis in the laboratory. The results of the examinations showed that European foul brood and American foul brood were the diseases of bees which attracted most interest in the State.

Bacillus alvei was found to possess a number of flagella arranged peritrichic instead of one flagellum at a pole, as Harrison at first reported, but later accredited Cowan with the statement (p. 56). The fact that *Bacillus alvei* was supplied by more than one flagellum had already been pointed out by Lambotte.

Concerning *Bacillus "X"* (*Bacillus larvæ*) the following is found:

It is a slender rod with moderate motility having a tendency to form in chains. The formation of spores and the arrangement of flagella is somewhat similar to that found in *B. alvei*. While *B. alvei* grows quite well on all the artificial media commonly used in the laboratory, the growth of *Bacillus "X"* is not so easily obtained. The medium which is most successful in the cultivation of this species is the one made from the larvæ of bees. Growth has been obtained with difficulty upon ordinary agar and gelatin.

The so-called palsy or paralysis received some attention, but after beginning this work it was soon realized that before it could be done satisfactorily it would be necessary to know something of the normal bacterial flora of the healthy bee. A brief study of the bacterial species most frequently found within and upon the normal bee was therefore made.

WHITE, JUNE, 1905.

About the time that this last report was published, a manuscript embodying all of the work done on bee diseases at the New York State Veterinary College for the State of New York was prepared as a thesis.[1] Since the manuscript is available to but few, it will not be reviewed here. With very few changes this manuscript was published as Technical Series No. 14, Bureau of Entomology, United States Department of Agriculture (p. 76).

WILSON, 1905.

Of course it is very frequently impossible on account of inadequate descriptions to identify certain organisms. In the case of *Bacillus alvei* there is but little excuse for any mistake, since the description which Cheyne has made is entirely adequate for this purpose. In this connection a paper by Wilson[2] is of interest.

He used a culture for demonstration purposes in a medical school, which he isolated from the tonsils of a patient with suspected diphtheria and identified it as *B. alvei*. He claims that *B. alvei* is fre-

[1] White, G. Franklin. June, 1905. The bacterial flora of the apiary with special reference to bee diseases. Thesis, Cornell University, Ithaca, N. Y.

[2] Wilson, Dr. R. J., 1905. Morphological characteristics of the *Bacillus alvei*. Proceedings of the New York Pathological Society, vol. 5, pp. 79–81.

quently seen in cultures from the throat. Now, it may be that Wilson made a correct identification, but inasmuch as the source of the culture was the throat, he should have been very careful about making the identification positive.

It might be mentioned here that not a few bee keepers have been startled by an announcement that *B. alvei* is found in human sputa. Some of them have reasoned, very naturally, that if all reports were true the sputum might be the source of foul-brood infection, but there is no convincing evidence, of course, that such is the case.

Burri, January, 1906.

Burri's next paper[1] was on "foul brood" and "sour brood." His discussion of foul brood is quite similar to that which appeared in his former paper (p. 64). We shall therefore direct attention at this time to that portion devoted to "sour brood."

The origin of the name "sour brood" is indefinite. Quoting from C. P. Dadant, an American writer, Burri writes that there are three diseases of the brood recognized in America—foul brood, sour brood, and black brood. This view would make sour brood synonymous with pickled brood, but as it will be learned later in his work on sour brood, Burri was studying for the most part at least European foul brood.

In his work Burri did not find a uniformity in the diseased brood examined either in the gross or the miscroscopic appearance. In one sample he found no bacteria, although the outward appearance of the larvæ indicated disease. In another sample the gross appearance did not suggest foul brood, and there were absent the bacteria which are commonly found in the disease; and in their stead there were present millions of bacteria which to the investigator did not seem to stand in etiological relation to the disease. In still a third instance the larvæ gave no outward sign of being killed by the bacteria of foul brood, but when studied culturally, they showed the presence of a very large number of unidentified bacteria together with a few of those which frequently accompany "foul brood." These findings illustrate, he says, some of the difficulties which are encountered in a study of the brood diseases bacteriologically.

Putting aside all samples which were unquestionably "foul brood," he attempted to group the remaining ones according to certain characteristics observed in a study of the gross appearance of the diseased brood. One character which seemed to be emphasized was the sour odor emitted by certain samples. On account of this he classified this condition as "sour brood." In testing the odor of brood dead of the disease, Burri recommends the holding of the nose

[1] Burri, Dr. R., January, 1906. Bakteriologische Untersuchungen über die faulbrut und Sauerbrut der Bienen. Pp. 39, pl. 1. Vorwort by U. Kramer.

very near the combs, or, better, the removal of a larva and testing it. He calls attention to the fact that "foul brood" (American foul brood) and "sour brood" (European foul brood) have probably often been confused by bee keepers of little experience and placed under one name, "foul brood."

Another point of difference between "foul brood" and "sour brood," as pointed out by Burri, is in relation to the consistency of the dead larvæ in the two conditions. In "foul brood," he says, a uniform ropy mass is all that remains of the decaying larva dead of the disease, while in "sour brood" the chitinous covering of the decaying larva permits its removal as a whole from the cell.

Besides the odor and consistency of the dead brood, Burri refers to the color as a third characteristic that serves to aid in the differentiation of "foul brood" and "sour brood." He writes that the larvæ of "foul brood" are cream colored soon after the development of the bacteria has begun, but later are a pale coffee brown, and finally a dark brown. In "sour brood," he says, the larvæ become discolored. At first they are a dirty yellow. The dry scales are less black than those of "foul brood."

Burri received samples which were reported to him to be "black brood." The older larvæ seemed to be affected and the microscopic and cultural examinations gave negative results. This strongly suggests that this is not the condition to which the term "black brood" has been referred in America. No conclusion was reached by him as to the cause of this trouble. Certain differences were noted by Burri between the descriptions by Dadant of the different brood diseases and his own observations. It is not difficult to understand why such differences should exist when one recalls that so many descriptions of the brood diseases in the past by Americans have been based largely upon faulty work.

Further on in his paper, Burri gives the microscopic findings and describes the gross appearance of a few larvæ taken from each of the eight samples of sour brood which he examined. He mentions in "sour brood" the yellowish color of the larvæ, the uncapped cells, and the presence of rather long rods. Short rods were also found, resembling in morphology *Bacterium güntheri*. On account of this similarity, in recording the presence of this latter species, Burri has referred to it as the "*güntheri*-forms." These facts concerning the gross appearance of the microscopic findings in "sour brood" suggest strongly that the condition is the same as the foul brood of Cheshire and Cheyne (European foul brood).

In summing up the results of his study on "sour brood," Burri emphasizes two observations: First, that there is a form of disease found all over Switzerland which possesses the characters mentioned for "sour brood"; and, second, that in the condition there is a certain

uniformity in the microscopic findings. There were medium-sized and small bacterial rods present together with forms resembling in morphology *Bacterium güntheri*. There was an absence of spores and of the corresponding vegetative forms. It was observed that one group of bacteria may predominate in some samples and another group may predominate in others. Where rods of relatively large size were found in brood which in gross appearance resembled sour brood, it was supposed that a double or mixed infection of foul brood and sour brood was present. This double infection, it was believed, occurred very frequently.

In continuing his bacteriological study of "sour brood" Burri encountered a few rather interesting species. *Bacillus alvei* was present in many samples of "sour brood" examined. From most of the samples examined difficulty was encountered in obtaining cultures of the microorganism to which he refers as the *güntheri*-forms. He reports, however, that this difficulty had been overcome and that he had obtained pure cultures of this species. He made some comparisons between the cultures of this species and those of *Bacterium güntheri* which resulted in the conclusion that while there was a certain relationship existing between them, the two were not the same.

Burri sums up the results of his study of "sour brood" as follows:

1. There is a disease of the brood accompanied by a rapid growth of bacteria, which have no direct relation with the bacteria of foul brood.

2. The larvæ attacked are characterized by the following symptoms: (*a*) More or less noticeable sour odor; (*b*) comparatively pale, dirty yellow color; and (*c*) a great resistance of the chitinous covering which allows the dead larva to be lifted intact from the cell as a moist mass.

3. In microscopic examination the contents of these larvæ are characterized by the presence of forms resembling sour milk bacteria (*güntheri*-forms) beside medium-sized and small rods. It is characterized also by the absence of large spore-bearing rods and spores.

4. Pure culture experiments with such bacterial material give proof of a certain relationship between the true sour milk bacteria and the *güntheri*-forms. The cultures also show that the medium-sized and small rods are strong acid producers. The name "sour brood" is therefore entirely justified.

With respect to the occurrence of "foul brood" and "sour brood" in the same colony one finds the following in Burri's paper:

In describing each attempt to isolate the sour brood *güntheri*-forms the rarely expected fact was demonstrated that in a whole series of cases, a growth of colonies of *Bacillus alvei*, the easily cultivated producer of stinking foul brood, was obtained from typical sour broody cells instead of the *güntheri*-forms desired. The series of cases of this kind could be greatly increased. Moreover, in the course of my investigations such findings have been repeated such a surprising number of times that I was forced to think there must be some close connection between the two diseases. For some time I was even inclined to believe that the sour brood bacteria represented only a certain stage of development in the foul brood bacteria but gave up this view when the morphological question was explained by means of culture experiments. To-day it can safely be affirmed that foul brood bacteria, sour brood *güntheri*-forms, and the

other types of rods found in sour brood cells are independent organisms, each with its own cycle of development. If various pathogenic bacteria are met with in a disease, medical men speak of the condition as a mixed infection. It seems that generally in sour brood we have to deal with such a mixed infection. As already pointed out, I have, occasionally in the microscopic examination, but particularly in the cultural tests of the comb material sent in, encountered the mixed infection of sour brood and foul brood so regularly, that I scarcely expect to meet with a case of pure sour brood. By this I mean a comb with sour brood cells in which at the same time foul brood germs are not to be found. This presumption, however, proved not to be true, for the specimen from Kaltbrunn must be considered as a case of "pure" sour brood. The first specimen from Murten which similarly gave the impression at first of being "pure," had to be considered subsequently to be foul brood, for the second specimen from the same source showed unquestionably the presence of *Bacillus alvei*.

The samples containing dead brood, which Burri studied from May, 1903, to September, 1905, were grouped under four headings, viz, "sour brood," "stinking foul brood," "nonstinking foul brood," and "dead brood free from bacteria."

In summing up Burri's work on "sour brood" the following interesting facts might be mentioned:

1. The origin of the term "sour brood" is not definite.

2. Burri considered three gross characters to be of especial value in the diagnosis of "sour brood"—a sour odor, a lack of ropiness of the decaying larvæ, and a dirty yellow color of the brood recently affected.

3. In "sour brood" were found a large number of short rods which resemble, on microscopic examination, *Bacterium güntheri* found in sour milk, and with these he found other rods of medium and large size.

4. When cultures were made from the larvæ dead of "sour brood," the *güntheri*-forms did not grow as a rule, but in their stead cultures of *Bacillus alvei* appeared sometimes in pure culture.

5. The cultivation of the *güntheri*-forms is reported as having been successful.

6. Burri believed that "sour brood" and the "stinking foul brood" are usually found together. This was suggested to him by the frequent presence of *Bacillus alvei* and the *güntheri*-forms in the same diseased colony. "Sour brood" was reported to have been found alone in one instance.

7. He grouped the samples of comb which contained dead brood into four conditions, viz, "sour brood," "stinking foul brood," "non-stinking foul brood," and "dead brood free from bacteria."

8. The true menace to bees he believed to be due to a bacillus which is difficult to cultivate.

We are not inclined to agree with all the views expressed by Burri in his work on "sour brood." The condition referred to as "sour brood" and "stinking foul brood" are probably but one disease, European foul brood; the "non-stinking foul brood" is the same as is now known as American foul brood, and the samples which were reported as containing no bacteria together with those which were

received labeled "black brood" were in most instances very probably the so-called pickled brood.

This completes for the present the consideration of the investigations made by Dr. Burri. His work is executed with much care, and his results are correspondingly valuable. For this reason we feel that anything which he writes on bee diseases can be recommended to the bee keepers for careful study.

Maassen, June, 1906.

Several interesting papers on bee diseases have been written by Maassen, of Dahlam, Germany. The first paper[1] to be considered is on "foul brood."

Of the samples received from 119 apiaries, 112 were found upon examination to be diseased. Of these 112 samples which were declared diseased, *Bacillus alvei* was found in only 13.

Maassen fed colonies large amounts of cultures of *Bacillus alvei* in both the vegetative and spore form during the brood-rearing season without producing the disease. An attempt was also made to inoculate the brood directly, but negative results were obtained by this method (p. 59). The conclusion was therefore drawn that *Bacillus alvei* had not the significance in brood infection that had ordinarily been attributed to it. In all cases where *Bacillus alvei* was not found there were other spore-bearing species observed. The presence of one species is especially emphasized which offered much difficulty in cultivation on the usual media of the laboratory (p. 60). This species he refers to as *Bacillus brandenburgiensis*. No definite proof was obtained of a causal relation between this spore-bearing species and the disease.

It seemed to Maassen at this time that spore-bearing bacteria were probably only secondary invaders in this disease condition. He was strengthened in his belief by the finding of what he supposed was a protozoan to which he gave the name *Spirochæte apis*. In all brood affected with the disease he records the presence of this microstructure. It was yet to be determined, he says, whether this last finding bore any causal relation to the disease in which it was found.

In this paper by Maassen the following points are of special interest:

1. Maassen was examining samples of brood which were suspected by the bee keepers to be "foul brood."

2. He does not mention two forms of "foul brood."

3. He found *Bacillus alvei* in 13 samples of "foul brood" out of 112 samples diseased.

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[1] Maassen, Dr. Albert, June, 1906. Faulbrutseuche der Bienen. Mitteilungen aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft. Heft 2, pp. 28-29.

4. He found in all the samples of foul brood examined, in which *Bacillus alvei* was absent, another species present which offered difficulties in its cultivation on artificial media and refers to the species as *Bacillus brandenburgiensis*.

5. He reports this species to be present in some of the samples, together with *Bacillus alvei*.

6. He used a large amount of the culture of *Bacillus alvei* in the inoculation of healthy bees and did not produce disease.

7. "Foul brood" was not produced with pure cultures of *Bacillus brandenburgiensis*.

8. He was inclined to the belief that bacteria are secondary invaders in "foul brood."

9. He believed that this view was strengthened by the finding of a microorganism to which he gave the name *Spirochæte apis*.

10. He reports this microstructure present in all samples of the disease which he had examined up to that time.

MAASSEN, JUNE, 1906.

Another paper appeared by Maassen,[1] in which he briefly refers to a disease which he says is known to the bee keepers as "stone brood."

The condition, he says, is characterized by the hard, leathery, brittle, odorless, and mummylike masses into which the larvæ and pupæ of bees are transformed with no marked change in their form. Accompanying the condition is a higher death rate among the adult bees.

The peculiar change in the brood was attributed to a fungus that grows well at a warm temperature, and whose characteristics when studied in pure cultures were found to be similar to those of *Aspergillus flavus*. The method of transmission of this germ was not determined. According to the observations that were made it was supposed that bees were very susceptible to the disease. This was especially true if the temperature was high or the hive was badly ventilated, and it was therefore recommended that these conditions be avoided in the treatment of the disease. Maassen expresses the belief that "stone brood" has often been referred to by bee keepers as "black brood," "new bee disease," "bee pest," and "pickled brood."

We are not familiar with the condition "stone brood," and we are not aware of its presence in America. The symptoms given do not correspond to those observed in the so-called black brood or in the pickled brood that are met with in this country. It is intimated in Maassen's paper that a publication on the mycotic diseases of bees was being prepared.

[1] Maassen, Dr. Albert, June, 1906. Die Aspergillusmykose der Bienen. Mitteilungen aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft. Heft 2, pp. 30-31.

Bahr, 1906.

Another publication by Bahr [1] appeared in 1906, in which he gives the results obtained from his further investigations. He reports that more than 200 cases of foul brood had been examined. The following points are noted in Bahr's paper:

1. One can not be sure with what disease he was working.
2. He does not always find *Bacillus alvei* in foul brood.
3. With cultures of *Bacillus alvei* he was not able to produce foul brood either by spraying the larvæ or by feeding cultures of the bacillus. He failed also to produce the disease by using the contents of the dead larvæ for spraying or as food in sugar sirup.
4. He suggests that the reason for these negative results may be either that *Bacillus alvei* is not the cause of foul brood or that the proper time or manner in which such infection can be produced experimentally had not been discovered.
5. He did not find any other bacillus as a possible cause of the disorder. *Bacillus alvei* was not found in the eggs or in the sexual organs of the queen, as had been reported by Cheshire (p. 21), Harrison (p. 49), and others.
6. He suggests that possibly the cause of the disease is an ultravisible virus and that possibly the disease is transmitted through the queen.

It appears likely that Bahr was working with European foul brood, but this is not at present positively known. If he studied American foul brood, he must have overlooked the fact that there are numerous spores (*Bacillus larvæ*) present in the decaying remains of the larvæ which do not grow on the artificial media commonly used. In support of his theory that the disease is transmitted by the queen he says that he has introduced a queen from a diseased colony into a healthy one and produced foul brood as soon as the queen could lay the eggs, and that he has introduced queens from healthy colonies into apparently doomed ones with the result that the diseased colonies quickly recovered.

These experiments should be repeated for a confirmation of the results. If, as is probable, Bahr worked with European foul brood, there were probably other factors present which were not accounted for. His failure to find *Bacillus alvei* in all the samples examined is interesting, and his failure to produce foul brood with cultures of *Bacillus alvei* repeats the experience of some others.

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[1] Bahr, L., 1906. Om Aarsagen til Bipesten og dennes Bekæmpelse. Foredrag holdt ved DBF's Diskussionsmøde d. 2 Septbr. 1906 i Esbjerg. Særtryk af Tidsskrift for Biavl. Nr. 17.

PHILLIPS, OCTOBER 3, 1906.

In 1906 a brief circular [1] was issued by this bureau giving the symptoms and treatment of the two brood diseases. This paper is of interest at this time only because it was the first occasion for the use of the names "American foul brood" and "European foul brood" in a publication of the bureau.

Since the name "black brood" had been, on account of an error, applied (p. 45) to the foul brood which Cheshire and Cheyne (p. 25) described, the name "black brood" was no longer needed. The name "foul brood," however, was being applied by the bee keepers (p. 60) to a disease which was clearly different from the foul brood described by Cheshire and Cheyne. This latter disease, therefore, needed a name. The laws that were in existence in some of the States at that time provided for the inspection of apiaries in which foul brood was found. In order that these laws could be interpreted, in accordance with their intent, to cover the brood diseases of an infectious nature, the name "foul brood" was retained in the names of these two brood diseases. To distinguish the two diseases by name, the adjective "European" was selected for the disease which had been early creditably studied by a European (p. 29) and the adjective "American" was selected for the disease which had been studied by an American (p. 62). These names were chosen only after consultation with a number of the leading bee keepers in America, who agreed that the names were well chosen.

The words "American" and "European" were not chosen to suggest a geographical distribution of the two diseases, as the opinion was held that both diseases exist in Europe as well as in America. Concerning the selection of these names the facts were emphasized in the preface of a paper to be discussed later (p. 76).

ERNE, NOVEMBER, 1906.

In 1906 Dr. Erne,[2] of Freiburg, Germany, reviewed Burri's work on the brood diseases and gave the results of his own investigations. Erne, too, obtained negative results in an attempt to produce "foul brood" with a culture of *Bacillus alvei*. This species was not found by him in 64 samples of "foul brood" received from different parts of Germany. For these reasons he expresses a doubt concerning any etiological relation between the species and the disease as found in Germany. He found, however, in all samples of the disease a bacterium which he thought probably was identical with the one which

[1] Phillips, E. F., October 3, 1906. The brood diseases of bees. U. S. Department of Agriculture, Bureau of Entomology, Circular No. 79. Pp. 5. (Superseded by Farmers' Bulletin 442, U. S. Department of Agriculture, "The treatment of bee diseases.")

[2] Erne, Dr. November, 1906. Bakteriologische Untersuchungen über die Faulbrut und die Sauerbrut der Bienen. Die Europäische Bienenzucht, pp. 148–151.

Burri observed to be difficult of cultivation. As this species was not obtained in pure culture, no inoculation experiments were made with it. By feeding foul-brood material to ten colonies, however, Erne proved that the disease with which he was working was infectious, since in every case typical foul brood was produced which contained the same bacillus previously observed.

To make clear his position, Erne summarizes as follows:

1. Burri has not furnished proof that sour brood is a contagious disease and that the bacterium described by him is the cause of the same.

2. It is not proven that there is more than one foul brood germ.

3. I consider as the cause of the epidemic foul brood causing the greatest destruction at the present time, a bacillus which I have found in all of my investigations, which can not be cultivated on the usual media, and which may perhaps be identical with the bacillus that Burri found to be difficult of cultivation.

In Erne's paper the following interesting facts are noted:

1. He was working probably only with American foul brood.

2. Erne took exception to the methods used by Burri in the attempt to obtain pure cultures of the bacillus which was found difficult of cultivation.

3. He emphasizes the importance of the experimental inoculations of healthy colonies in the demonstration of the cause of a disease of bees.

4. He did not find *Bacillus alvei* in 64 samples of foul brood examined from Germany.

5. He obtained negative results when healthy bees were fed pure cultures of *Bacillus alvei*.

6. He questioned an etiological relation between *Bacillus alvei* and "foul brood."

7. He demonstrated the infectiousness of foul-brood material by the production of "foul brood" in healthy colonies.

8. He met with a species of bacterium in foul brood which was difficult to cultivate on artificial media.

9. He considered this germ to be the cause of foul brood, although the fact was not demonstrated.

10. Erne did not in his study of "foul-brood" material meet with a microorganism corresponding to *Spirochæte apis*.

While Erne does not devote much time to bee-disease investigations, his writings show that considerable care is exercised in his work. The bee keepers, therefore, will be profited by reading any papers written by this author.

White, November 6, 1906.

In 1906 the manuscript mentioned on page 67 was published as a bulletin.[1] In the preface the reason for the selection of the names

[1] White, G. F., Ph. D. November 6, 1906. The bacteria of the apiary, with special reference to bee diseases. U. S. Department of Agriculture, Bureau of Entomology, Technical Series, No. 14. Pp. 50.

"European foul brood" and "American foul brood" for two of the infectious diseases of the brood of bees is explained.

The technique used by the writer of the bulletin in making the investigations is given in Part I. In this portion also is discussed somewhat the normal flora of the apiary. It was not the intention in making this study of the normal flora to give a complete list of the bacteria which might be encountered, but to study those species which occur most frequently, and to describe them with sufficient care to make their identification possible.

The results of the study indicate that comparatively few bacteria are present in healthy colonies, on combs, in honey, in larvæ, or on adult bees. In the intestine of adult bees, however, there were usually found a very large number of individual bacteria, which, as a rule, however, represented comparatively few species. One species, an anaërobe, is of much interest since it occurs quite constantly and in very large numbers. It might be mentioned that the bees that did not show this intestinal flora were usually the younger adults. A number of fungi and yeasts were also encountered.

The subject-matter in Part II, "The diseases of bees," is not materially unlike that which appeared in earlier publications to which references have already been made. The author of the paper under consideration had reached no definite conclusion concerning the etiological relation of *Bacillus alvei* to European foul brood, the disease in which this species is usually found in large numbers. That any direct causal relation did exist seemed questionable.

In American foul brood, *Bacillus larvæ* was found in large numbers in the larvæ dead of the disease in all the samples examined. Pure cultures of the organism had been obtained, but not in a suitable form for making inoculation experiments. The author of the paper did not feel justified in stating positively that *Bacillus larvæ* is the cause of the disease. All that seemed justified was the statement that the organism had been found constantly present in the disease.

The following brief summary was made of the results obtained from the study of the bee diseases:

(1) There are a number of diseased conditions which affect the apiary.

(2) The disease which seems to cause the most rapid loss to the apiarist is European foul brood, in which is found *Bacillus alvei*—first isolated, studied, and named by Cheshire and Cheyne in 1885.

(3) The distribution of *Bacillus alvei* in the infected hive is as follows:

(*a*) The greatest number of infecting germs are found in the bodies of dead larvæ.

(*b*) The pollen stored in the cells of the foul-brood combs contains many of these infecting organisms.

(*c*) The honey stored in brood combs infected with this disease has been found to contain a few bacilli of this species.

(*d*) The surface of combs, frames, and hives may be contaminated.

(*e*) The wings, head, legs, thorax, abdomen, and intestinal contents of adult bees were found to be contaminated with *Bacillus alvei*.

(*f*) *Bacillus alvei* may appear in cultures made from the ovary of queens from European foul-brood colonies, but the presence of this species suggests contamination from the body of the queen while the cultures are being made and has no special significance.

(4) The disease which seems to be most widespread in the United States we have called American foul brood, and the organism which has been found constantly present in the disease we have called *Bacillus larvæ*. This disorder was thought by many in this country and other countries as well to be the foul brood described by Cheshire and Cheyne, but such is not the case.

(5) From the nature of American foul brood it is thought that the organism has a similar distribution to that of *Bacillus alvei*.

(6) It appears that European foul brood was erroneously called "New York bee disease" or "black brood" by Dr. William R. Howard in 1900.

(7) There is a diseased condition affecting the brood of bees which is being called by the bee keepers "pickle brood." No conclusion can be drawn from the investigation so far as to the cause of the disease.

(8) *Aspergillus pollinis*, ascribed by Dr. William R. Howard as the cause of pickle brood, has not been found in this investigation and is not believed by the author to have any etiological relation to the so-called "pickle brood."

(9) Palsy or paralysis is a diseased condition of the adult bees. No conclusion can yet be drawn as to its cause.

(10) Formaldehyde gas, as ordinarily used in the apiaries, is insufficient to insure complete disinfection.

Maassen, February, 1907.

In 1907 Maassen[1] reported on his work of the preceding year on foul brood. Samples were received from 100 apiaries. An examination gave evidence of disease in 79 of them. Disease was not found in the other 21. "*Spirochæte apis*" was reported in samples from 67 apiaries. Accompanying it *B. brandenburgiensis* was reported in 66 cases and *B. alvei* in one. *B. alvei* was not found generally in the samples from Germany, occurring only in 11 of the cases.

Among the 100 samples examined there were 2 in which was found a species in almost pure cultures which before had been found accompanied by *Bacillus alvei*. This species Maassen named *Streptococcus apis*. He says that it belongs to the pneumococcus group, being different from other members of the group by its marked peptonizing character. Upon a certain medium he reports that the species could be cultivated very easily. In 10 cases in which *B. alvei* was found *Streptococcus apis* was reported in 8. No conclusive results were obtained in his attempts to demonstrate the relation between any of the organisms and the disease condition.

In his report the following points of special interest are noted:

1. Maassen did not express any suspicion that two distinct infectious diseases might be present in the condition he was studying as foul brood.

2. He reports the presence in samples from 67 apiaries of a microorganism which he had previously named *Spirochæte apis*, and with

[1] Maassen, Dr. Albert, February, 1907. Über die sogenannte Faulbrut der Honigbienen. Mitteilungen aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft. Heft 4, pp. 51–53. 6 figs.

it he finds associated *Bacillus brandenburgiensis* in 66 cases and *Bacillus alvei* in one case.

3. He found *Bacillus alvei* in 11 cases of diseased brood. The majority of these samples probably were from apiaries affected with European foul brood.

4. He observed and cultivated a species which he named *Streptococcus apis*. This species, he states, belongs to the pneumococcus group and is easy of cultivation. In 10 samples in which *Streptococcus apis* was found *Bacillus alvei* was found in 8.

5. He states that he had not reached a final conclusion concerning the relation between the microorganisms and the disease encountered.

IMMS, JUNE, 1907.

The Board of Agriculture and Fisheries of Great Britain requested Mr. A. D. Imms, of Christ College, Cambridge, to make a study of the cause and nature of a disorder among bees. References to this disorder have been made in the last f w years as the Isle of Wight disease. Imms [1] made a report on his work in 1907.

From this report an idea of the rapid losses which were attributed to the disease can be obtained. It is stated that the disease was so prevalent that it seemed almost impossible to keep a colony healthy for 12 months. Seventy colonies were reduced to 8 in two years. One bee keeper lost 20 colonies out of 22. Three other bee keepers in the same district lost their entire apiaries, consisting of 12, 8, and 4 colonies, respectively. Another bee keeper lost over 50 colonies and about a dozen other bee keepers had no bees left.

Imms gives the following in his description of the symptoms of the disease:

> The earliest noticeable symptom of the disease is the inability of the affected bees to fly more than a few yards without alighting. As the disease progresses, the bees can only fly a few feet from the hive and then drop and crawl about aimlessly over the ground. They are often to be seen crawling up grass stems, or up the supports of the hive, where they remain until they fall back to the earth from sheer weakness, and soon afterwards die. In a badly infected stock great numbers of bees are to be seen crawling over the ground in front of the hives, frequently massed together in little clusters, while others remain on the alighting board. If the hives be opened, numbers of diseased individuals will be often met with inside. They are found clustered together around the queen and show very little inclination for movement until disturbed and are entirely unable to fly. Badly diseased individuals show very little inclination for stinging; those that are less severely attacked often sting very actively.
>
> If a badly diseased bee be carefully examined it will be seen to have lost its power of flight, and it crawls about with the hinder extremity of the body dragging on the ground; frequently it walks about with its wings "out of joint," the hind wings protruding obliquely upwards and above the anterior pair. The only other external symptom of the disease is seen in the abdomen, which is frequently distended beyond

[1] Imms, A. D., June, 1907. Report on a disease of bees in the Isle of Wight. Journal of the Board of Agriculture, Vol. XIV, No. 3, pp. 129–140, 4 figs.

its normal proportions. This distension, however, is not by any means constant, and was chiefly noticed in the case of the native bee; in the half-breed with the Italian bee, with its longer and slightly more slender abdomen, no unusual distension could be observed.

The disease appears to differ from what is usually termed "bee-paralysis," in that the infected individuals do not exhibit the characteristic black and shiny appearance, and neither I myself, nor any bee keepers who have paid attention to the disease, have observed the curious trembling motion of the limbs and body which is regarded as a symptom of that disease.

The disease appears to be entirely confined to the adult bees, the brood remaining unaffected. I have conducted a microscopical examination of a large number of eggs, larvæ at all stages of development, and pupæ, and have failed to detect anything of a pathological nature among the brood. All had the characteristic pearly white appearance of healthy specimens although belonging to a badly infected hive. The eggs were undergoing development and showed not the slightest trace of discoloration or shriveling, the larvæ were healthy in every way and were coiled up in their normal attitude, and nothing wrong could be detected with the pupæ or the newly hatched bees.

In describing the "Nature of the disease" Imms writes in part as follows:

> The disease is eminently one of the digestive system and might be described as being a condition of enlargement of the hind intestine. Over 150 diseased bees have now been examined and all have been found to exhibit the same symptoms.

The author states that the bacteriological work on the disease was in progress. The work which had already been done demonstrated the presence of a large number of bacterial rods. No conclusion was reached as to the cause of the disease, nor had any remedy been found in the treatment that was successful in the hands of all bee keepers.

Some of the more important points in the paper might be summarized as follows:

1. The disease, so far as was determined, was of recent origin.
2. The disorder described seemed to be very rapidly fatal to adult bees. The brood seemed to be unaffected.
3. To Imms the trouble seemed to be neither dysentery nor the so-called paralysis.
4. No conclusion was reached as to the cause of the disorder.
5. No treatment was demonstrated to be successful.

White, July 29, 1907.

On July 29, 1907, there was issued a circular[1] briefly describing some experiments which demonstrated for the first time the cause of American foul brood. Although spores had been observed in very large numbers in the larvæ dead of this disease, no satisfactory medium had yet been devised by which pure cultures could be obtained that were suitable for purposes of experimental inoculations.

[1] White, G. Franklin, July 29, 1907. The cause of American foul brood. U. S. Department of Agriculture, Bureau of Entomology, Circular No. 94. Pp. 4.

The way by which this difficulty is overcome is reported in the publication under consideration. Young pupæ were used in making the medium. These were picked from a comb containing healthy brood, crushed, strained through cheesecloth, and then diluted by adding water equal to from 20 to 50 times the volume of the crushed brood used. This solution was then passed through ordinary filter paper and subsequently through a Berkefeld filter. In this way a sterile filtrate was obtained. About 2 c. c. of the sterile filtrate was then added by means of a sterile pipette to liquefied agar which had been cooled to 45° or 50° C. If pure cultures were desired, agar tubes thus prepared were inoculated with a small amount of diseased brood and plates were poured. If, however, culture growth was desired for the inoculation of bees or experimental animals, it was obtained from these specially prepared agar tubes by first inclining them and then securing the growth by inoculating the surface of the inclined agar with a pure culture of *Bacillus larvæ* obtained from the plates. At no time was this special medium to reach a high temperature.

Two colonies were now fed the scales of American foul brood, suspended in sirup. American foul brood resulted from these inoculations with symptoms the same as are found in an apiary in which the disease appeared through the natural means of infection. Similar results were reported by Erne (p. 76). These experiments were sufficient to prove that American foul brood can be produced experimentally by feeding; also, that the scales of the disease contained the virus.

Having demonstrated the fact that American foul brood can be produced by feeding and having obtained pure cultures of *Bacillus larvæ* in suitable form for inoculation purposes, the next step to be taken, very naturally, was to inoculate healthy colonies with pure cultures of *Bacillus larvæ*. This was now done, and as a result of such inoculations American foul brood was produced with symptoms identical with those produced when the scales were used in feeding. The decaying brood in the disease thus produced contained the large number of spores that are always found in brood dead of this disease, and from the diseased material pure cultures of *Bacillus larvæ* were obtained.

The results obtained from these experiments in which pure cultures of *Bacillus larvæ* were used in making the inoculations justified for the first time the statement that American foul brood was caused by a specific microorganism.

It seemed to the author of the circular that probably the species which had given different workers considerable difficulty in cultivation, in many cases at least, was nothing other than *Bacillus*

larvæ. The "microorganism" named *Spirochæte apis* by Maassen (p. 72) was shown to be giant whips which have their origin in the growth of *Bacillus larvæ*.

Phillips, December 31, 1907.

In connection with the study of American foul brood it was noticed that the scales formed by the drying down of the dead larvæ are not destroyed if the comb becomes infested with either of the two wax moths. These observations were recorded in a publication[1] of this bureau. Sometimes it is desirable to have the dried scales of American foul brood in large quantities. These can be easily obtained free from the comb by allowing a well dried and badly diseased sample to become infested with wax moths.

Maassen, 1908.

Another paper[2] by Maassen appeared in 1908. In his former publications this author has dealt with only one form of foul brood. In this paper, however, he states that two forms of the disease have been known for many years, a "mild" form and a "virulent" one.

Maassen's description of the gross appearance of the brood affected with the "mild" form is similar to that given by Dzierzon (p. 18) and others. The disease therefore is quite probably European foul brood. This view is further strengthened by the bacteriological examinations which he reports. His description of the "virulent" form is also similar to that given by Dzierzon (p. 18) and others. The condition is most likely, therefore, American foul brood.

Following the discussion of these two forms of "foul brood" Maassen discusses the etiology of "foul brood." He expresses the belief that foul brood is a disease of the digestive apparatus of the larvæ and can be produced by various causes. As producers of "foul brood" *Bacillus alvei*, *Streptococcus apis*, and *Bacillus brandenburgiensis* are mentioned by him. Besides these three species he reports the presence in the diseased brood of a species of yeast and spore-bearing bacilli. *Bacillus alvei* and *Streptococcus apis* are reported to have been found in both forms of foul brood, while *Bacillus brandenburgiensis* was found in only one of them.

In that form of the disease in which uncapped brood seemed mostly to be affected, Maassen reports the presence of *Bacillus alvei* in 51 samples out of the 53 examined. When *Bacillus alvei* predominated in the sample, he interpreted the odor as being more "sweat-like" in character than when *Streptococcus apis* was in predominance; and

[1] Phillips, E. F. December 31, 1907. Wax moths and American foul brood. U. S. Department of Agriculture, Bureau of Entomology, Bulletin No. 75, Part II. Pp. 19–22.

[2] Maassen, Dr. Albert, 1908. Zur Ätiologie der sogenannten Faulbrut der Honigbienen. Arbeiten aus der kaiserlichen biologischen Anstalt fur Land- und Forstwirtschaft. Bd. VI, Heft I, pp. 53–70. 2 pls.

when the latter species predominated the odor was likened to that of sour paste. In samples from two apiaries Maassen failed to find *Bacillus alvei,* but found *Streptococcus apis* in large numbers. The two cases in which *Bacillus alvei* was absent were suspected of being the sour brood referred to by Burri (p. 68). Maassen was inclined to believe that the latter condition is more widespread in Switzerland than in Germany. In 41 samples of the 51 containing *Bacillus alvei,* the species was accompanied by *Streptococcus apis.* The relative number of *Bacillus alvei* and *Streptococcus apis* varied.

The "*guntheri*-forms" mentioned in Burri's paper (p. 69) are very probably the species to which the name *Streptococcus apis* Maassen has been applied. Maassen expresses a similar belief. The following description of *Streptococcus apis* is an abbreviation of the one by Maassen.

Occurrence.—This species is found in "foul brood," occurring most frequently in that form in which the larvæ when attacked are uncapped.

Morphology.—In form it is not perfectly spherical but is a lancet-like, pointed coccus that appears as either a Diplococcus or a Streptococcus in the body of the larvæ as well as in artificial media. A capsule is present.

Gram's stain.—The organism is not decolorized by gram's method.

Oxygen requirements.—It grows aërobically as well as anaërobically.

Bouillon.—The medium becomes at first turbid, and later a deposit forms at the bottom of the tube. Reaction is but little changed.

Glucose, lactose, saccharose, galactose, levulose, and mannite bouillons.—Increased growth takes place in these bouillons with the formation of acid.

Agar slant.—A thin iridescent growth takes place. The condensation water is clouded with a sediment present.

Blood serum.—There is a perceptible growth. The colonies are droplike. No liquefaction of the medium takes place.

Potato.—The organism grows well on this medium.

Milk.—Growth takes place rapidly. After 24 hours the casein is coagulated and later some of the coagulum peptonizes.

Gelatin.—After about 40 hours at 20° C. a whitish-gray growth is observed with a beginning liquefaction of the medium.

Indol.—Indol is not formed.

Nitrates.—Nitrates remain unchanged.

Disinfectants.—This species proved very resistant to drying. After three-fourths of a year of drying the organism was not dead.

Burri (p. 70) met with some difficulty in the cultivation of the species to which he referred as the *güntheri*-forms. In a few cases, Maassen apparently had some difficulty also with his *Streptococcus apis,* but in most cases no difficulty was encountered. The difficulty,

Maassen says, was obviously due to an acid which was present. He likened the condition to that obtained in cultures in those artificial media in which a sugar is present. Maassen suggests that the death of *Streptococcus apis* under these conditions is not without significance if this organism is the cause of the disease.

The feeding of pure cultures of *Streptococcus apis* to healthy colonies gave negative results. He reports that negative results were obtained also when healthy colonies were fed larvæ containing the cocci with no *Bacillus alvei* present. When similar larvæ, however, were fed to healthy bees together with a suspension of the spores of *Bacillus alvei* he reports that the disease was produced.

Having considered the etiology of the "mild" form of foul brood (European foul brood), Maassen took up for consideration the cause of the so-called "virulent" form of the disease (American foul brood). This latter disease, he says, is far more widespread in Germany than is the former. From 347 samples of diseased brood examined in five years, 294, almost 90 per cent, were affected with the "virulent" form of the disease. In this form he usually found *Bacillus brandenburgiensis*. This species was so named by him because his first experience with it was in a sample from the Province of Brandenburg, Prussia. Maassen describes the morphology and cultural characters of *Bacillus brandenburgiensis*. He says that this species is the cause of the foul brood most commonly found in Germany.

Maassen also says that he found spirochæte-like forms (p. 73) in the unstained decaying "foul-brood" mass. He considers them a good diagnostic agent in the virulent form of the disease. He says further that in the progress of his investigations he found *Spirochæte apis* to be nothing more than tufts of the flagella of *Bacillus brandenburgiensis* (p. 82). He also says that after great difficulty two media were found on which *Bacillus brandenburgiensis* grew well. One was agar made from bee larvæ (p. 62), and the other was agar made from the brains of calf or of pig. Maassen reports that he has produced disease by feeding cultures of *Bacillus brandenburgiensis*. Each colony fed received the cultures from 10 to 20 tubes. When considerable culture was fed the disease appeared in from 6 to 10 days after the feeding of the colony. He states that the disease is present in America, and that a bacillus has been found in it which has been named *Bacillus larvæ*.

Maassen has therefore confirmed most of the facts stated in the paper (p. 80) which was received by him at least four months before his was published.

The following is a brief summary of Maassen's paper:

1. He mentions that he has encountered in his studies two forms of foul brood. These were described by Dzierzon and others.

2. The form which seems to be European foul brood he refers to as the "mild" form; and the other one, which seems to be American foul brood, he refers to as the "virulent" form.

3. In the "mild" form he finds *Bacillus alvei* and *Streptococcus apis*, together with a few other species.

4. In two instances he did not find *Bacillus alvei* present, but did find *Streptococcus apis*. This condition he believes to be the "sour brood" of Burri.

5. In a few cases only he reports difficulty in obtaining cultures of *Streptococcus apis* on artificial media.

6. In the disease (American foul brood) which is most common in Germany, Maassen finds a species which he calls *Bacillus brandenburgiensis*.

7. In this disease he also finds *Bacillus alvei* generally associated with *Bacillus brandenburgiensis*.

8. In a few samples he found *Streptococcus apis* accompanying *Bacillus brandenburgiensis*.

9. In his experimental inoculations, he obtained positive results when *Bacillus brandenburgiensis* in either the vegetative or spore form was fed to a healthy colony.

10. He obtained negative results when healthy colonies were fed cultures of *Streptococcus apis*.

11. He failed to produce disease by feeding to the healthy colonies dead brood containing *Streptococcus apis* but no *Bacillus alvei*.

12. He reports positive results when the spores of *Bacillus alvei* were fed together with dead brood containing *Streptococcus apis*.

Maassen, September, 1908.

Maassen[1] published another paper in 1908. He reviews briefly the history of the study of foul brood. An attempt was made to learn of the distribution of the diseases in Germany. For this purpose letters were sent to bee keepers in various parts of the country, making inquiry into the disease conditions of the apiaries. Answers were received from two States. He obtained data, however, from other sources which caused him to believe that foul brood is frequent in almost all of the German States. A review is then made of his own investigations relating to foul brood.

White, December 26, 1908.

The object of a paper[2] read before the National Bee Keepers' Association on October 14, 1908, was to direct the attention of bee

[1] Maassen, Dr. Albert, September, 1908. Uber die unter dem Namen "Faulbrut" bekannten seuchenhaften Bruterkrankungen der Honigbiene. Mitteilungen aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft. Heft 7. Pp. 24. 4 Tafeln. July, 1909. 2 Auflage. Pp. 31. 4 Tafeln.

[2] White, G. Franklin, Dec. 26, 1908. The relation of the etiology (cause) of bee diseases to the treatment. U. S. Department of Agriculture, Bureau of Entomology. Bulletin No. 75, Part IV, pp. 33-42. Reprinted. Annual Report of the National Bee Keepers' Association for 1908. Pp. 60-65. U. S. A.

keepers to the important relation which exists between the etiology of a disease and its rational treatment.

The author at first deemed it advisable to direct the attention of his hearers to a consideration of the nature of disease. A brief discussion is then given of the etiology of diseases, illustrating the statements made mainly by citing phenomena observed in bee diseases. In discussing the etiology, the usual division into predisposing and exciting causes is made. Of the predisposing causes of diseases it seemed well to mention age, sex, heredity, race, climate, and preexisting disease, inasmuch as these factors may be active in one or more of the diseases of bees. Of the exciting causes of disease, food and microorganisms are the only ones mentioned, since food, bacteria, protozoa, and fungi have been thought by one writer or another to be the direct exciting cause of bee diseases. A brief reference is then made to the nature of bacteria, protozoa, and fungi.

Mention is made in the paper of the fact that a microstructure had been encountered in the investigations of European foul brood which had failed to grow on artificial media. This was referred to as "*Bacillus Y.*" Some hope was entertained that it might sometime be proved to be the exciting cause of the disease. The great resistance exhibited by the spores of *Bacillus larvæ* toward disinfectants was emphasized by citing some preliminary experiments.

The following are some of the facts to which the attention of the bee keepers was directed:

1. Disease is nothing more than a departure from a state of health.

2. The departure is the result of some cause.

3. The cause is, as a rule, a combination of factors which constitute what is known as the etiology. Age, sex, race, and climate seem to figure as predisposing factors in bee diseases. Bacteria, protozoa, and fungi have all been studied as probable exciting causes. Bacteria are the only kind of a microorganism that has been proven to be the cause of a bee disease.

4. Comparatively little is known of the etiology of bee diseases—a statement which, as one becomes familiar with the diseases of other animals and man, is found to be true for them also.

5. The exciting cause of but one bee disease is positively known.

6. A rather interesting microstructure was encountered in European foul brood which had refused to grow when sown upon artificial media. This is referred to as "*Bacillus Y.*"

7. A treatment, either preventive or curative, can best be devised only after the cause is determined.

8. Before treating a disease, a diagnosis is advisable. This can most accurately be done by knowing the cause and finding it in the diseased body.

9. The conclusion drawn is that in a knowledge of the causes of bee diseases lies hope for their control.

MALDEN, FEBRUARY, 1909.

In 1909 Dr. Malden, of Cambridge, England, made a report[1] on his investigations of a disease which appeared on the Isle of Wight. A paper by Imms (p. 79) discussing this disorder has already been considered.

Malden went to the island in May, 1908, and by interviewing the bee keepers and inspecting colonies found that the disease had apparently quite died out. The disease had been seen, however, in March and early April of that year. After a short period of apparently complete absence the disease again appeared about the middle of June, 1908.

Malden states that as a rule the disease causes greater losses during the summer than in winter. The reverse, however, has been noted at times. May and June are according to most observers the months during which the disease is usually most rapidly fatal. Infected colonies are not always destroyed. They may recover but are subject to a later attack by the disease.

Malden's investigations into the cause of the disease include a study of the gross and microscopical anatomy of the diseased bee, together with a bacteriological study of it. In his bacteriological study one species was encountered to which, on account of its resemblance to *Bacillus pestis*, the supposed cause of bubonic plague, he gave the name *Bacillus pestiformis apis*. The morphology, cultural characters, and pathogenic properties of this bacillus are given as follows by Malden:

It is an aerobic, non-motile, Gram negative, non-acid-fast, short, broad bacillus, varying in its morphological appearances upon different media. No flagella could be demonstrated. On *agar* it grows fairly well, forming in twenty-four hours medium-sized (largest 0.1 cm. in diameter), round, white or slightly yellowish, smooth, glistening, flattened, dome-shaped colonies. On further growth the colonies do not increase much in size, and unless very thickly sown they show little tendency to coalesce. After twenty-four hours' growth the bacilli are of medium length (1–1.5μ), broad, and with distinctly rounded ends. Many of them are distinctly oval. They have a tendency to stain better at the ends than in the middle (polar staining). Occasionally the lightly staining central portion appears as a distinct band, especially when the organism is lightly stained. After seven days' growth very little general change is noticed, though a few large involution forms make their appearance. On *gelatin* growth takes place rapidly in the form of colonies, resembling those produced on agar. The organisms are more rounded than on agar, being distinctly oval in shape, and polar staining is not so marked. On *potato* a considerable raised cream coloured growth is produced in twenty-four hours at 37° C., which continues to spread. The bacilli are larger than when grown on agar, but the light central band is not quite so

[1] Malden, Walter, M. A., M. D., February, 1909. Further report on a disease of bees in the Isle of Wight. Journal of the Board of Agriculture, Vol. XV, No. 11, pp. 809–825.

well defined. When stained by Neisser's method, a few show polar bodies (metachromatic granules). Involution forms, many of which grow to a large size, appear rapidly, and are very abundant after forty-eight hours' growth. In *broth* at 37° C. a cloudy growth is first formed, but later the medium becomes clearer, and a considerable yellowish, flocculent deposit is produced. A surface film is usually seen after a few days' growth, and may be very marked. If the tube is shaken, the film sinks, or is broken up, but another forms. The bacilli resemble those found on gelatin cultures. No acid or gas is produced in media containing *glucose, lactose, saccharose, dulcite, mannite, maltose, dextrin, or glycerine.*

Pathogenic Properties.—A single infection experiment was made with a culture of this bacillus. A healthy stock of bees was placed in a hive in a green-house. After a few days all the openings were closed with muslin, and the bees fed on syrup. When the bees had become accustomed to this treatment, broth cultures of the bacillus were mixed with the syrup. Within a few days considerable numbers had died, and specimens of apparently diseased bees showed the bacilli in their chyle stomachs, which also showed the fragile condition found in naturally infected bees. Distention of the colon could not be taken as a diagnostic point, as this condition was found to be present in healthy specimens of this stock taken from the hive before the experiment was started. The majority of the bees showed no signs of disease a week after feeding was commenced.

The results of the anatomical and bacteriological studies of this disease by Malden are clearly set forth in the following quotation from his paper:

Anatomically the majority of diseased bees show great distention of the colon, and a fragile condition of the chyle stomach. In many obtained from diseased stocks, and apparently suffering from the disease, distention of the colon is absent. All the organs, except those just mentioned, are normal. Healthy bees confined to their hives for a few days very closely resemble diseased bees in regard to the condition of their intestinal canals. It is impossible, therefore, both from the clinical and anatomical points of view, to diagnose whether any given bee is suffering from the disease or not.

Histologically the chyle stomach appears to be the only organ affected, and bacteriologically plague-like bacilli were frequently encountered in it, in some cases apparently within the epithelial cells. These bacilli were not found either in the brood of diseased hives or in the chyle stomachs of healthy bees. For these reasons I am inclined to regard these organisms as the cause of the disease. I am, however, well aware that I have not fully established their relationship to the disease, since I have not been able to demonstrate them in every case either microscopically or by culture, or to find, except in very advanced cases, any very definite lesions constantly associated with their presence. I feel that my inability to discover any means of cultivating the organism with certainty even from chyle stomachs, in which it was present in abundance as shown by microscopical preparations, constitutes the most serious difficulty in establishing its relationship to the disease. By their morphology alone, few pathogenic bacteria can be recognized, since morphologically indistinguishable, but non-pathogenic, organisms are frequently encountered. Consequently, until some satisfactory cultivation methods have been discovered, the bacteriological diagnosis of this organism must in most cases remain in doubt, for organisms simulating it in morphology probably exist.

Concerning this disease and its cause the following points are to be emphasized:

1. Malden found the disease which Imms reported still present on the Isle of Wight.

2. The evidence obtained indicates that the disease is infectious.

3. An organism was encountered in a number of diseased bees, to which Malden gave the name *Bacillus pestiformis apis*.

4. This organism was not proven by Malden to be the cause of the disease.

ZANDER, AUGUST, 1909.

In 1909, Dr. Zander, at Erlangen, Germany, wrote an interesting paper [1] concerning the cause of a disease affecting the adult honey bee.

From his studies Zander was led to believe that there are two forms of dysentery. One form he considers to be noninfectious and comparatively harmless. The cause of this form is attributed to various conditions, such as disturbance of the colony, queenlessness, improper winter stores, deficient opportunity for a cleansing flight, etc. A second form of dysentery Zander refers to as the malignant form. In referring to the virulence of this form Zander says that it has all the characteristics of an infectious disease, destroying more colonies in one spring in the neighborhood of Erlangen than foul brood had during the entire preceding year in the whole State of Bavaria.

During his investigations in 1907 Zander found in the mid-gut of diseased bees a protozoan to which the name *Nosema apis* was given. This portion of the intestine of all the bees which died of the "virulent" form of dysentery was found to be milk-white and completely filled with Nosema spores. Queens from dysenteric colonies were examined and found also to be infected with the protozoan. No drones were found infected. This was supposed to be due to the fact that there are no drones during the active dysenteric season. The excrement from the bees also contained numerous spores.

Zander expresses the belief that the transmission of the disease is made possible by the deposit of excrement on the frames and walls of the hive, which takes place when no opportunity is afforded the bees for a cleansing flight. The mutual feeding practiced by bees hastens, it is suggested, the spread of the infection. The bees are supposed to be subjected to further infection from without on account of the spores that are spread about through the medium of the excreta of the flying bees. Robbing also is given as a fruitful means of transmission. The use of contaminated combs from apiaries in which the infection is present is also thought to be a means for the spread of the disease.

The prognosis is supposed to depend somewhat upon conditions. Long, hard winters are given as a cause of very heavy losses with this disease. If, on the other hand, the spring is warm with a good flow

[1] Zander, Dr. Enoch, August, 1909. Tierische Parasiten als Krankheitserreger bei der Biene. Münchener Bienenzeitung, Heft 9. Pp. 11.

and the old bees are rapidly replaced by young healthy ones, the young bees remain healthy unless a second infection takes place. It is suggested that if this second infection takes place it asserts its presence about four weeks after the first outbreak. It is then commonly thought by the bee keeper to be a different disease, the one to which the name "May disease" is sometimes applied. In the so-called "June disease" Zander reports the presence of infection with *Nosema apis* also.

Zander performed some inoculation experiments for the purpose of demonstrating the relation of *Nosema apis* to the "virulent" type of dysentery. He describes one in which infected material was fed in honey to a colony free from disease. The excrement from bees affected with dysentery, together with bees so affected, was ground and added to diluted honey. The mixture was filtered and put into two combs, and the combs were placed into a queen-right colony, which had been examined and found to be free from disease. Three days later the bees began to die with all the symptoms of "May" and "June" disease. Many dead and dying bees were found in the yard in the direction of flight of the bees. A microscopic examination demonstrated the presence of *Nosema apis* in these bees. After eight days the mid-gut of most of the diseased bees was milk-white. The colony became weaker and weaker, and at the end of a month only a handful of bees remained.

Zander concludes from his work that *Nosema apis* is the exciting cause of the infectious form of dysentery.

The following is a brief summary of Zander's first paper on *Nosema apis* and the disease with which he found it associated:

1. Zander discovered a protozoan that attacks the epithelial cells of the mid-gut of the adult honey bee. To this protozoan was given the name *Nosema apis*.

2. He discusses dysentery of bees under two forms—a mild form and a virulent one.

3. He believes the mild form to be noninfectious and probably due to a number of different causes; the infectious or virulent form he believes to be due to *Nosema apis*.

4. He is inclined to believe that this infectious form is the same disorder as the one to which "May disease" and "June disease" has frequently been applied.

5. It should be noted that Zander does not claim to be working with a new disease, but is simply seeking to determine the cause of dysentery—a disease with which most bee keepers have had experience.

Maassen, March, 1910.

Another paper [1] by Maassen appeared in 1910.

At this time the content of the intestinal tract of bees taken from colonies affected with different brood diseases was made the subject of study.

When bees were examined that were taken from colonies suffering from "sour brood," Maassen reports the presence of *Streptococcus apis*. This species thrives well, he says, in the intestine, and its power to grow upon artificial media is not lost (p. 84). Likewise, he reports that the spores of *Bacillus alvei* will develop and the organism grow in the intestine of adult bees. Confined bees, it is stated, showed the presence of these two species for weeks in the intestine.

In the case of American foul brood, Maassen reports that the spores of *Bacillus brandenburgiensis* do not germinate in the intestine of the adult bee, nor do the vegetative forms multiply there. He reports that after a few days a noticeable decrease is to be observed in the number of spores present. These observations caused Maassen to caution those treating bee diseases against the probability of infection from these germ carriers.

The paper also contains a report on the samples received for diagnosis. Material was received from 85 apiaries. When examined, 66 of the samples gave evidence that disease was present. Forty-five are reported as American foul brood, one as a mixed infection of American foul brood and European foul brood, 10 as European foul brood, and 10 as a mixed infection of European foul brood and sour brood.

Here the following points are to be noted:

1. Maassen reports that *Bacillus alvei* is found in the intestine of adult bees taken from a colony affected with European foul brood and that this species multiplies in this locality.

2. He reports that *Streptococcus apis* is found in the intestine of bees that are taken from colonies affected with sour brood and that this species also multiplies in this locality.

3. He reports that the spores of *Bacillus brandenburgiensis* do not increase in the intestine of the adult bee.

4. These germ carriers, Maassen suggests, must not be overlooked in devising methods of treatment.

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[1] Maassen, Albert, March, 1910. Untersuchungen über die Epidemiologie der sogenannten Faulbrut der Bienen. Mitteilungen aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft, Heft 10, pp. 37-39.

Maassen and Nithack, March, 1910.

Simultaneously with the paper just considered there was published a paper [1] on bee dysentery by Maassen and Nithack. Dead adult bees from entirely isolated localities were received and examined. There was a history of supposed poisoning accompanying the bees. No cause for their death, however, could be found. It is recorded that no *Nosema apis* was found.

The first dysentery observed by these men was in two colonies taken from different apiaries. One was a queenless two-frame nucleus and the other a queen-right colony of six frames. These two colonies were transferred to wire cages. After about three weeks symptoms of dysentery were observed. At the beginning of the spotting *Nosema apis* was not found in the excrement, but could be demonstrated in the mid-gut. Several days later, when the intestine showed the appearance described by Zander, the parasite was found in the intestine.

These findings caused Maassen and Nithack to confine in wire cages a series of small queenless colonies. These cages were kept in a room whose temperature ranged from 14° to 16° C. The bees were obtained from different sources, and all chances of becoming infected from food, hives, or combs subsequent to being taken into the room were excluded. The results obtained from this experiment were similar to those of the preceding one. Other experiments somewhat similar were performed.

These men report that in many colonies in which no visible signs of dysentery were present there were found bees containing *Nosema apis*. They believe that among bees this protozoan is widely distributed. Up to the time of their writing they had not failed to find it in colonies that were suffering from dysentery.

Malden, June, 1910.

In 1910 Malden in a paper [2] gave a good brief review of the status of the present knowledge of bee diseases. He gives some further observations concerning the Isle of Wight disease. Referring to *Bacillus pestiformis apis* (p. 87) he writes in part as follows:

> This organism may frequently be found to have penetrated between the cells of the lining membrane of the chyle stomach and to be present in large numbers in the loosened tissue behind the secreting cells. It has been found present in about 60 per cent. of all the bees affected with this disease which have been examined. * * * It appears highly probable that this organism is the cause of the disease, but up to the present time no infection experiments have been successful in producing the complaint in healthy stocks, so that its relation to the disease cannot be said to be proved.

[1] Maassen und Nithack, March, 1910. Über die Ruhr der Bienen. Mitteilungen aus der kaiserlichen biologischen Anstalt für Land- und Forstwirtschaft, Heft 10, pp. 39–42.

[2] Malden, Walter, M. A., M. D., June, 1910. Diseases of bees. Reprinted from The Journal of Economic Biology, Vol. V, Pt. 2. Pp. 41–48.

Malden reports that the disease had within the previous two years spread from the Isle of Wight to the mainland (England). There seems to be evidence that the virulence of the disease is less than when first observed on the Isle of Wight.

Zander, 1910.

Zander in 1910 in a publication [1] gives a good brief review of the work that has been done on the cause of the brood diseases. The disease in which *Streptococcus apis* is found Zander refers to as "sauerbrut"; the disease which he refers to as "faulbrut," we call American foul brood; and we diagnose as European foul brood what he refers to as "brutpest."

Zander, 1911.

In 1911 Zander writes [2] of the diseases and enemies of the adult bees. The morphology, life history, and distribution of *Nosema apis* (p. 89) had been the subject of further study by him and in this publication he gives the results of his work.

A BRIEF CHRONOLOGICAL SUMMARY OF THE WORK ON THE CAUSES OF BEE DISEASES.

THE DIFFERENT DISEASES THAT ATTACK BEES.

That bees suffer from disease is recorded in the writings prior to the Christian era. It is not known, however, which diseases are referred to.

Schirach (p. 13) in 1771 classified the diseases of bees. In his classification he mentions, among other diseases, dysentery and foul brood.

Just when and by whom it was first recognized that foul brood was of two forms is not known. It would seem that Schirach, in 1771, observed that foul brood was not always the same. One finds that Leuckhart (p. 14) in 1860 and Molitor-Mühlfeld (p. 14) and Preuss (p. 15) in 1868, all indicated by their writings that they considered foul brood to be of more than one form.

Dzierzon (p. 18), in his "Rational Beekeeping" in 1882, describes two forms of foul brood. The descriptions of the two forms agree very closely with those of European foul brood and American foul brood.

Cheshire (p. 19) began to write early in the year 1884 of two forms of foul brood, but before the close of the year he (p. 22) declared that there was only one form.

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[1] Zander, Dr. Enoch, 1910. Die Faulbrut und ihre Bekämpfung. Part I. Handbuch der Bienenkunde in Einzeldarstellungen. Mit 4 Tafeln und 8 Abbildungen nach Originalen des Verfassers. Pp. 31.

[2] Zander, Dr. Enoch, 1911. Krankheiten und Schädlinge der erwachsenen Bienen. Part II. Handbuch der Bienenkunde in Einzeldarstellungen. Mit 8 Tafeln und 13 Abbildungen grösstenteils nach Originalen des Verfassers. Pp. 40.

During the following decade there continued to be different opinions entertained as to the classification of the infectious brood diseases.

Many bee keepers were convinced from their experience with the diseases of the brood that there existed two distinct infectious disorders (p. 60). By a more careful study of these diseases it has been shown positively that the brood is attacked by more than one infectious disease.

These diseases as understood by the writers of this bulletin are briefly discussed on pages 11 and 12.

The classification of the adult bee diseases is yet very unsatisfactory.

THE CAUSES OF BEE DISEASES.

As exciting causes of bee diseases different workers have from time to time suggested different agents.

Schirach (1771) (p. 13) suggested two causes for foul brood—improper food as one, and a fault of the queen as a second.

Leuckart had at first inclined to the view that the infectious foul brood was due to a fungus, but from his observations made in 1860 (p. 14) he arrived at the conclusion that this was not true.

Molitor-Mühlfeld (1868) (p. 15) attributed the cause to a parasitic ichneumon fly, *Ichneumon apium mellificarium.*

Preuss (1868) (p. 15) and Schönfeld (1873–74) (p. 16) were inclined to believe that an infectious form of foul brood was due to a fungus, to which the former gave the name *Cryptococcus alvearis.*

Cheshire (1884) (p. 21) and Cheyne (1885) (p. 34) were inclined to believe that foul brood was due to a bacillus, *Bacillus alvei.*

The same disease which Cheshire and Cheyne studied came to the attention of William R. Howard in 1900 (p. 46), and he declared that the cause of it was a bacillus, to which he gave the name *Bacillus milii.*

Recent work (p. 81) has proven that American foul brood has as an exciting cause a specific bacillus, to which the name *Bacillus larvæ* has been given.

The writers of this bulletin believe that the causes for the other bee diseases have not as yet been satisfactorily demonstrated.

INDEX.

	Page.
American foul brood, cause	80–82
description	11
name given	75
Aspergillus flavus	73
pollini, supposed cause of "pickled brood"	42–44
Bacillus A	51, 57
alvei, description by Cheyne	31–33
Harrison	49–50
name given	21
brandenburgiensis (see also *Bacillus larvæ*).	
name given	72
depilis, use of name	24
gaytoni, use of name	24
larvæ (see also *Bacillus X* and *Bacterium X*).	
name given	77–78
mesentericus vulgaris, identity	53–57
milii, identity	47
supposed cause of "black brood"	45–47
pestiformis apis, description	87–88
pestis	87
thoracis, supposed connection with "black brood"	46
X (see also *Bacterium X* and *Bacillus larvæ*).	
name used	67
Y, name used	86
Bacterium güntheri	69–71
X (see also *Bacillus X* and *Bacillus larvæ*).	
name used	63
Bahr, work on bee disease	64, 74
"Black brood" (*see also* European foul brood).	
name given	44
Blow-fly, experiments thereon	17, 20
Burri, work on bee disease	64–66, 68–72
Calliphora vomitoria, experiments thereon	17, 20
Camphor treatment for bee disease	51
Carbolic acid treatment for bee disease	21, 22, 40
and tar treatment for bee disease	51
Cheshire, work on bee disease	18–25
and Cheyne, work on bee disease	25–29
Cheyne, work on bee disease	29–35
and Cheshire, work on bee disease	25–29
Cowan	56
Creolin treatment for bee disease	51
Cryptococcus alvearis, supposed cause of "virulent foul brood"	16, 17
fermentum	16
Dysentery	13
Dzierzon, work on bee disease	18
Erne, work on bee disease	75–76
Eucalyptus treatment for bee disease	51
European foul brood, description	12, 44
name given	75

Page.
Formaldehyde gas treatment for bee disease 62, 63
Formic acid treatment for bee disease 51
Foul brood, American. (*See* American foul brood.)
European. (*See* European foul brood.)
Güntheri—forms 69–71
Harrison, work on bee disease 48–53, 56–58
Howard (William R.), work on bee disease 41–47
Ichneumon apium mellificarium, supposed cause of bee disease 15
Imms, work on bee disease 79–80
Isle of Wight disease 13
description 79
Lambotte, work on bee disease 53–56
Larvæ and pupæ of bees used in making culture media 20, 55, 62–63, 81
Leuckart, work on bee disease 14
Lortet, work on bee disease 38–39
Maassen, work on bee disease 72–73, 78–79, 82–85, 91
and Nithack, work on bee disease 92
Mackenzie, work on bee disease 39–41
McLain, work on bee disease 35–38
Malden, work on bee disease 87–89, 92–93
Molitor-Mühlfeld, work on bee disease 14–15
Moore and White, work on bee disease 58–61
Musca vomitoria. (See *Calliphora vomitoria*.)
Naphthalene treatment for bee disease 51
Naphthol-beta treatment for bee disease 39, 40, 51
"New York bee disease" (*see also* European foul brood).
name given 44
Nithack and Maassen, work on bee disease 92
"Nonstinking foul brood," same as American foul brood 71
Nosema apis, name given 89
Paralysis 13
Phillips, work on bee disease 75, 82
"Pickled brood," description 12
name given 42
Pieris rapæ. (See *Pontia rapæ*.)
Pontia brassicæ, experiments thereon 17
rapæ, experiments thereon 17
Preuss, work on bee disease 15–16
Salicylic acid treatment for bee disease 40, 51
Schirach, work on bee disease 13–14
Schönfeld, work on bee disease 16–17
"Sour brood," 68
same as European foul brood 71
Spirochæte apis, name given 72
"Stinking foul brood," same as European foul brood 71
"Stone brood" 73
Streptococcus apis, description 83
name given 78
Thymol treatment for bee disease 51
White, work on bee disease 61–63, 66–67, 76–78, 80–82, 85–87
White and Moore, work on bee disease 58–61
Wilson, work on bee disease 67–68
"X brood" (*see also* American foul brood).
name used 63
Zander, work on bee disease 89–90, 93

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